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Workshop 2.1

Measuring Brain Activity by Using Imaging Methods

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In this lecture, I will discuss about various approaches to measure brain activity by using imaging methods. I will begin by giving a historical perspective on the available methods in measuring brain activity. I will compare each imaging method and discuss their advantages and disadvantages. I will also try to highlight the future upcoming technologies. I will aim that the students will be able to choose the most suitable imaging technology for their experimental work by the end of my lecture.

Workshop 2.2

Advanced Microscopy Techniques in Neuroimaging

Alp Can

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Pioneering works by 19th-20th century neuroscientists such as T. Schwann (1810-1882), M.J. Schleiden (1804-1881), C. Golgi (1842-1926) and S. Ramón y Cajal (1852-1934) enabled us to understand the basics of neurons and neuronal circuits. In parallel to their discoveries, these foremost researchers also challenged microscope manufacturers to strive for continual innovation which is still valid today; the ever-growing field of neuroscience constantly demands microscope developers to innovate and renovate new microscopy techniques to resolve the crucial scientific questions. Current research suggests that the human brain consists of about 86 billion neurons with 125 trillion synapses in the brain cortex alone. Human brain is by far the most complex organ in the body and one of the most complex biomachines we have ever known, typically consuming somewhere around 70% of the total energy a body generates. Since the human brain is a large organ but its organization makes use of very tiny connections, one of the biggest challenges we face in microscopy for neuroscience is scale. Additionally, it is not always possible to extract structural and functional information at the same time, which means that often there is no "ideal" imaging system. Thus, complementary microscopy techniques frequently need to be combined with and supported by methods from, for example, cell biology or biochemistry. From the invention of oil immersion in 1878 up to today's phase contrast, DIC, confocal and super-resolution microscopy, microscope developers have always been driving scientific discoveries through innovative microscopy techniques. The 21st century is widely recognized as the age of digitization. Powerful new computer technology and algorithms can now process vast amounts of raw data and lead to scientific discoveries. Automated wide field imaging systems allow for high-throughput multi-channel fluorescence screening of cell cultures, small organisms and histology samples, including data processing and archiving. Wide field microcopy today is both cost-efficient and easy to use with established protocols for a broad range of neuroscience applications. However, thick samples such as developing organisms, bran tissue or organoids can pose challenges as a result of the digital camera collecting additional light from above and below the focal plane. This is where modern point scanning confocal microscopes come into play, delivering high-contrast 3D imaging and advanced spectral imaging capabilities for methods. The development of new detector technologies extends the capabilities of confocal microscopy systems by introducing much greater light efficiency, resulting in gentle super-resolution imaging with high sensitivity. Light Sheet Fluorescence Microscopy (LSFM) also known as Selective Plane Illumination Micro-scopy/SPIM) is a conceptually new technology that virtually eliminates photo-damaging effects to the sample. LSFM is ideally suited to recording the neuronal development of living organisms

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such as zebrafish and to volume rendering of optically-cleared brain tissues. It can even visualize brain activity in 3D at single-cell level, using zebrafish calcium indicator dyes at maximum temporal resolution. For even higher structural resolution, scientists need to employ electron microscopy techniques. However, while brain organization is naturally three dimensional, these electron techniques are inherently limited to fixed sample tissues and 2D surface imaging. To overcome the latter limitation, various technologies have emerged over the past few years. These include automated array tomography workflows with integrated sample preparation and large area imaging as well as 3D volume reconstruction, enabling ultra-high-resolution datasets of whole brain sections while preserving the samples for further investigation and archiving. In all these techniques, we see that the future is multi-modal, with many imaging techniques being developed to take on new challenges as they arise.
*The author declares no conflict of interest with any microscopy hardware and/or software developer.

Workshop 2.3

Fluorescence Calcium Imaging: A Powerful Research Tool for Biomedical Science

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Ca^{2+} is a ubiquitous intracellular messenger that regulates multiple cellular physiological and pathological functions including muscle contraction, energy production, membrane excitability, neurotransmitter release, synaptic transmission, learning and memory formation, enzyme activity, fertilization and gene expression, as well as control of cell survival and apoptosis nearly in all organ systems. So using intracellular calcium changes/"calcium signals" as information carrier is a strategic approach. Fluorescence Ca^{2+} imaging is a powerful imaging technique which relies on detecting dynamic changes in free intracellular Ca^{2+} ion concentration, $[Ca^{2+}]i$ in living cells. Calcium signals can remain locally near the site of calcium entry into the cytoplasm but can also propagate over long distances and invade all around the cytoplasm. Kinetic and amplitude of calcium signaling should be considered when deciding on the specifications of the infrastructure; as one might miss a rapid and small amplitude calcium signal due to incapacity of the system or invest unnecessarily high for slow dynamic and high amplitude calcium signal to be study. In this technique the required infrastructure includes special calcium indicator dye bind one or more Ca^{2+} ions (both single wavelength and *ratiometric dyes* are available; among most popular ratiometric dye are fura-2, indo-1, ...), adequate light source (arc lamp or monochromators) required for excitation of the calcium indicator, a detector for detecting the emitted photon (imaging photon detectors (IPD)/ charge-coupled *device* (*CCD*) camera), a fluorescence microscope equipped with adequate objective (high NA value and adequate working distance) and software. *In vivo* Ca^{2+}-imaging method is also available and getting wider use. Thus, evaluation of intracellular Ca^{2+} signals, their temporal and spatial properties in isolated cells *in vitro* and *in vivo*, can be used to decipher physiological regulation and pathophysiology of the organ system, and for drug development approaches.

Workshop 3.1

The GnRH Pulse Generator

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The hypothalamic gonadotrophin-releasing hormone (GnRH) pulse generator that drives the pulsatile secretion of the gonadotrophic hormones, LH and FSH, is at the core of the reproductive system and yet the mechanisms underlying GnRH pulse generation are not fully understood. The discovery that humans and rodents with an inactivating mutation in kisspeptin or its receptor fail to progress through puberty or show normal pulsatile LH secretion, suggested that kisspeptin is a key regulator of pulsatile GnRH secretion. Attention has focused on the kisspeptin expressing neuronal population in the hypothalamic arcuate nucleus (ARC), the location of the GnRH pulse generator. These neurones, known as KNDy, because they co-express neurokinin B (NKB) and dynorphin A (Dyn) from a network in the ARC, but innervate the distal processes of the GnRH neurones at the level of the median eminence where they stimulate

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GnRH secretion. It is generally thought that NKB acting on its receptor (NK3R) functions as an excitatory signal to depolarise KNDy cells postsynaptically in the network, resulting in kisspeptin output to the GnRH neurones to initiate each GnRH pulse. The co-released Dyn functions as an inhibitory signal within the KNDy network, acting presynaptically on kappa opioid receptors (KOR) to inhibit the release of NKB, thus terminating kisspeptin release and ceasing the signal for GnRH secretion. I will present experimental approaches, including electrophysiological, optogenetic and fibre photometry that have helped our understanding of how the KNDy neural network drives pulsatile secretion of GnRH/LH.

Workshop 3.2

Mathematical Modelling in Neuroscience & Neuroendocrinology

Margaritis Voliotis

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Hormone rhythms are ubiquitous and essential to sustain normal physiological functions. Combined mathematical

modelling and experimental approaches have shown that these rhythms result from regulatory processes occurring at multiple levels of organization. Here, I will review how such an interdisciplinary approach has been successfully applied to unravel complex regulatory mechanisms in the reproductive axis that allow robust generation of LH pulses via means of a simple mathematical model. This mathematical model provides a quantitative framework for understanding the reproductive neuroendocrine system. Ultimately, the insight provided by mathematical models could lead to novel experimental tools able to continuously adapt parameters to gradual physiological changes and the design of clinical interventions to restore normal endocrine function.

Workshop 3.3

Experimental Models for Studying Polycystic Ovary Syndrome (PCOS) & Premature Ovarian Insufficiency (POI)

Aylin Yaba Uçar

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The global human population is rapidly increasing. However, despite the rapid growth of the world population, unfortunately, approximately 15% of couples cannot have children due to infertility. Despite advances in assisted reproductive technologies, infertility is still one of the major health problem worldwide. To identify the complex nature of these diseases, different animal models were created to mimic reproductive problems help us understand the underlying causes and mechanisms of reproductive dysfunction. Polycystic ovary syndrome (PCOS) and premature ovarian insufficiency (POI) are the common reproductive problems in women of reproductive age. PCOS is the most common endocrine disorder in women, is characterized by both reproductive and metabolic features. Another is that POI is a mysterious and complicated disorder which is a one of the main cause of infertility. POI relevance is continuously growing because of the increasing number of women desiring conception beyond 30 years of age and owing to the increased different source of inducers. Taken together, PCOS-like animal models and POI animal models provide unique opportunities to explore the underlying mechanisms governing follicular development and oocyte maturation competence. Although animal models have defined key signalling pathways, they have their limitations, use of appropriate animal models is enabling discovery, validation, and optimization of novel biomarkers and treatments for women with PCOS or POI. Furthermore, extensive studies can be design to develop new animal models approach leads to new infertility treatment strategies for PCOS and new markers for POI patients that predicts whether women are at risk of POI would, therefore, provide in early diagnosis and fertility treatment.

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Conferences

Conference 1

The Importance of Hormone Dynamics

Stafford Lightman

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Although the pulsatile nature of gonadotrophin secretion is well recognised, it is less well appreciated that almost all hormones (except for pro-hormones such as thyroxine) are secreted in a pulsatile manner, and that their receptor signalling systems have adapted to read this oscillating message. Cortisol (in man) and corticosterone (in the rodent) is no exception. It is released in hourly pules, and it is changes in the size of these pulses that creates the circadian rhythm se well described in the textbooks. This oscillating pattern of hormone secretion is created by a feedforward:feedback interaction between pituitary corticotropes and adrenal fasciculata cells and is a result of the feedforward delay that arises from the need to synthesise cortisol *de novo* from cholesterol in response to ACTH. I shall show how the pulsatile nature of plasma glucocorticoids determines optimal metabolic and cognitive responses both in the rat and in man. We have also gone on to develop a novel automated sampling system that allows us to measure interstitial fluid levels of hormones, metabolites and drugs up to every five minutes over the 24 hours. We have used this to establish normal patterns of multiple steroids and their dynamics over the 24 hours and have used this to compare with patients with Cushings disease, primary hyperaldosteronism and Addison's disease. We are also looking at whole tissue metabolome and believe this will offer important new data for diabetes mellitus, obesity, shift work related disease and for assessment of risk factors and personalised medicine. It also offer novel direct measurement of drug metabolism and kinetics at a tissue level.

Conference 2

Astroglia in Neuropsychiatric Disorders

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Astroglia are the homoeostatic cells of the central nervous system that control a normal function of synaptically connected neuronal networks and contribute to brain defence. Recent advances in studies of pathological potential of astroglia indicate that astrocytes are fundamental for most (if not all) neurological diseases. Neuropathological and neuroimaging studies demonstrate prominent astroglial atrophy and astroglial asthenia occurring in most of neuropsychiatric illnesses. In chronic diseases such as schizophrenia and major depression decrease in astroglial numbers and functional capabilities are, arguably, fundamental for pathological developments being responsible for neurotransmitter dysbalance and failures in connectivity within neural networks. It is therefore possible to hypothesise that neuropsychiatric diseases represent chronic astrogliopathology, which compromise glial homeostatic and defensive capabilities, and the degree of astroglial pathological changes define the progression and outcome of these disorders.

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Conference 3

Pituitary Dysfunction Due to Sport-Related Head Injury

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Traumatic brain injury (TBI) which is a public health problem worldwide and it is one of the most common causes of pituitary dysfunction. The main causes of post TBI pituitary dysfunction are car accidents, falls, violence and war accidents including blast-related brain injuries. Recent data revealed that hypopituitarism may also occur in athletes dealing with combat sports including boxing, kickboxing and football which are characterized by chronic repetitive head trauma and they are accepted as mild traumatic brain injury (mTBI) or concussion. The prevalence of pituitary dysfunction in athletes is reported to ocur in a range of 20 to 45 % and GH is the most common hormone lost. There is a significant negative correlation between peak GH levels and boxing duration, and between peak GH levels and number of bouts in boxers. On the other hand, pituitary volume in boxers with hypopituitarism is smaller when to compared to the boxers with normal pituitary function and healthy subjects. Repetitive head blows in athletes may result in disrupted blood-brain-barier and consequently results in release of some brain markers to the peripheral circulation. Release of hypothalamo-pituitary antigens to the circulation activates T and B cells and triggers synthesis of antipituitary antibody (APA) and anti-hypothalamus antibody (AHA) which may be responsible for the late development of pituitary dysfunction. Both boxing and kickboxing lead to impaired cognitive performance as revealed by P300 auditory event-related potentials. Apolipoprotein E polymorphism is related to the development of pituitary dysfunction in TBI victims including boxers and kickboxers. Prevention of TBI in athletes especially during childhood is crucially important and strategies for prevention should be specified to each sport including combat sports. Treatment of hypopituitarism with appropriate replacement of deficient hormones is beneficial in the improvement of symptoms and quality of life in athletes with sport-related brain injury.

Conference 4

Interactions between Sensory and Limbic Networks Drive State Transitions of Habenular Activity

Emre Yakşi

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The habenula (Hb) is a brain region with increasing popularity due to its strong link to addiction, mood disorders and prediction of outcomes. Recently, we demonstrated that the zebrafish Hb is innervated by mitral cell axons and exhibit prominent odor responses. Moreover, we showed that Hb neurons exhibit spatio-temporally structured ongoing (spontaneous) activity, which reflects internal states of the Hb networks. It remains still unclear, if/how odor responses and the ongoing activity of Hb neurons can modulate each other and how these interactions modulate Hb funciton. Our recent findings showed that ongoing Hb activity is mostly driven by the activation of limbic brain regions such as the zebrafish homologs of the amygdala (Dm) and the hippocampus (Dl). Moreover, we also showed that these limbic and the olfactory inputs are integrated in Hb in a non-linear fashion. Our results suggested that Hb lies in the heart of a brain wide network and act as “a hub” or “a switchboard”, which can regulate or gate the communication of sensory and limbic forebrain areas with the monoaminergic nuclei that control animal behavior.

Conference 5

Restoring Function of Inactivating Mutations of GPCRs in the Reproductive Hormone Axis

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Reproduction in vertebrates is driven by hypothalamic peptides, kisspeptin and neurokinin B which stimulate gonadotropin releasing hormone (GnRH), which in turn stimulates luteinizing hormone (LH) and follicle

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stimulating hormone (FSH) which regulate testis and ovarian function.

Inactivating mutations in G-protein coupled receptors (GPCRs) at all levels of this axis give rise to incomplete reproductive development and adult infertility. The majority of the mutations in these GPCRs cause misfolding of the receptor and a failure to traffic to the cell surface. We have therefore sought for cell permeant small molecules which can bind orthosterically or allosterically to stabilize the nascent GPCR in the endoplasmic reticulum and chaperone the mutant GPCR to the cell membrane.

We have successfully identified cell-permeant small molecules targeting receptors at all levels of the axis and demonstrated rescue of cell surface expression, receptor binding and signaling for inactivating mutations in NKB, GnRH, LH and FSH receptors.

These discoveries represent an advance towards personalized medicine for GPCR inactivating mutations in the human reproductive hormone axis. As GPCRs constitute 80% of signaling in humans, inactivating mutations are likely to be a major contributor of disease and hence targets for small molecule rescue of function.

Conference 6

Kiss1 Neurons as Hypothalamic Regulators of The Mammalian Reproductive Axis: Insights from Circuitry Mapping and Transcriptomics Profiling

William Henry Colledge and Stephen Manchishi

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Kisspeptin neuropeptides, encoded by the *Kiss1* gene, are key regulators of the mammalian reproductive axis and are required for both puberty and ovulation by stimulating GnRH release. *Kiss1* neurons are located in two main areas of the hypothalamus: the arcuate (ARC) region, which regulates basal GnRH pulsatility and the anteroventral periventricular (AVPV) region, which controls the preovulatory LH surge. Fundamental steps in understanding how the reproductive axis is co-ordinated with other physiological processes are an accurate description of the neuronal circuitry communicating with *Kiss1* neurons and molecular profiling to establish signalling pathways in the *Kiss1* neurons. We have used a Kiss-CRE mouse line to undertake conditional viral tracing with genetically modified tracer viruses to define afferent neuronal inputs to *Kiss1* neurons. Several of these neuronal populations have been implicated as physiologically relevant in controlling the reproductive axis. These include the suprachiasmatic nucleus, which communicates information about day length; the subfornical organ, which provides information about peripheral metabolic status; the amygdala, which responds to pheromone signals and POMC neurons in the ARC, which regulate feeding behaviour. To complement the circuitry mapping and to gain an insight into the potential physiological responses of *Kiss1* neurons, we have also performed transcriptomics gene expression profiling. RNAseq analysis of individually isolated and pooled *Kiss1* neurons from the AVPV and ARC regions have identified several differentially expressed genes between these two populations. Some of these differences were expected such as the Dynorphin and Neurokinin B signalling pathways in ARC *Kiss1* neurons. We have also identified novel pathway however, such as dopaminergic, serotonergic and drug addiction pathways in AVPV *Kiss1* neurons and relaxin, VEGF and prolactin signalling pathways in ARC *Kiss1* neurons. These data will be useful to fully understand the way in which *Kiss1* neurons are modulated by diverse physiological inputs.

Conference 7

What Starts Puberty? An Epigenetics Explanation: *KMT2D*

Ali Kemal Topaloğlu

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What controls the initiation of pubertal development remains unknown. The activity level of the hypothalamo-pituitary-gonadal (HPG) axis varies dramatically throughout the different phases of human life. While quiescent during childhood, at the time of puberty the HPG axis reawakens to induce secondary sexual characteristics and reproductive maturation. Epigenetics is postulated to be a determinant of pubertal onset. Indeed, it has been recently shown in rodents that the Trithorax Group (TrxG) and Polycomb Group (PcG) of epigenetic modifiers act at Kiss1 gene regulatory regions to turn the GnRH pulse generator ON or OFF respectively. Yet, the role of epigenetics in human puberty has not been shown to date. We recently detected rare sequence variants in

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KMT2D, a member of the TrxG, by screening a group of patients with Idiopathic Hypogonadotropic Hypogonadism (IHH), a condition featuring pubertal failure due to central causes. Inactivating mutations in *KMT2D* are normally known to result in Kabuki Syndrome, a multisystemic disorder clinically and pathophysiologically akin to CHARGE syndrome, which is due to mutations in a chromatin remodeler, Chromodomain helicase DNA binding protein 7 (*CHD7*). It may be that milder mutations in these two genes cause isolated IHH, in contrast, more deleterious mutations cause typical severe syndromic phenotypes. Based on the role of *Kmt2d* and other TrxG activators on rodent puberty and the preliminary data from 25 IHH patients who have evidently deleterious *KMT2D* mutations, it appears that *KMT2D* is part of an activating switch in the reawakening of the HPG axis at the expected time of puberty, thus its inactivating mutations result in IHH.

Conference 8

Exploring Novel Neuronal Pathways from the Retina to the SCN Using Transgenic Rat Models and Viral Transfection Systems

Mike Ludwig

Centre for Discovery Brain Sciences, University of Edinburgh, Edinburgh, UK

In all animals, the transition between night and day engages a host of physiological and behavioural rhythms. Retinal ganglion cells (RGCs) detect the ambient light level in the environment and these project to the suprachiasmatic nucleus (SCN) of the hypothalamus to entrain circadian rhythms that are generated within the SCN. Using transgenic rat lines, immunohistochemistry, tracer injection and viral transfection systems, we show here that vasopressin (VP) is expressed in many retinal cells that project to the SCN[1]. Using triple immunohistochemistry, we found that VP-RGCs co-expressed the vesicle glutamate transporter 2, and that 74% of VIP positive cells and 66% of GRP positive cells in the SCN were apposed by boutons from VP-RGCs fibres. VP-RGCs were often closely juxtaposed to immunoreactive melanopsin expressing cells and 25% co-expressed melanopsin. *In vitro* patch-clamp recordings from RGCs showed that 75% of these cells were transiently excited by light, and the other 25% were inhibited. Recording of the spike activity of single SCN neurons in urethane-anesthetised rats in vivo showed that two thirds of light-responsive cells were excited by light and one third were inhibited[2]. In a number cells excited by light, the light-induced activation was reduced by 30% after an icv injection of a vasopressin V1a antagonist. Microdialysis experiments have shown that either retino-hypothalamic tract stimulation or light evokes vasopressin release in the SCN. Light-induced vasopressin release also enhances expression of the immediate early gene product Fos in the SCN, which is critical for photic entrainment of circadian rhythms. Thus, these newly discovered vasopressin cells in the retina are a major, light-activated pathway that has a key role in regulating important circadian rhythms. The previously reported association of vasopressin with jet-lag raises the interesting possibility that interventions in vasopressin signalling from the retina may have important therapeutic benefits.

Conference 9

Effects of Graded Caloric Restriction

John R. Speakman

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The impact of reducing available calories on lifespan was discovered almost exactly 100 years ago. Yet despite intensive study for the past 40 or so years the mechanism that underlies the effect remains unclear. One problem is that during restriction many things change. It is therefore difficult to separate the things that mediate the impact on lifespan from things that are irrelevant consequences of the restriction. One important observation made several years back is that the impact on lifespan gets larger as the level of restriction increases – at least to about 65% restriction. This opens up a valuable approach because by exposing individuals to graded levels of restriction we can ore easily identify graded responses that potentially underpin the lifespan effect. We have conducted an expansive study of graded calorie restriction in mice. This has included monitoring multi-tissue transcriptomics, metabolomics, and proteomics, changes in body composition and aspects of whole animal and tissue physiology and behaviour. This has allowed us to develop a model for how caloric restriction works central to which is a neuroendocrine axis underpinned by the integrated activities of several

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neuropeptides. This model can be tested by knocking out these neuropeptides and exploring the responses to restriction. In this talk I will present the data we have already published to derive the model of how caloric restriction works, followed by new unpublished data from mice where two key genes have been knocked out – these are neuropeptide Y (NPY), and the melanocortin 3 receptor (MC3R). The responses to these manipulations and how they differ from what we predicted will allow us to refine the model in the future.

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Symposia

Symposium 1.1

Ghrelin and Reward

Suzanne L. Dickson

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Around 2006-2007, we found that ghrelin, an orexigenic gut-brain hormone, activates the mesoaccumbal midbrain dopamine pathway that confers reward. This pathway is known to be activated during approach behaviour for things that are salient/pleasurable, both natural (eg food) and artificial (eg alcohol). We hypothesised that by engaging this pathway, ghrelin may enhance food reward and food motivated behaviour and that its effects may extend to include other kinds of rewards. Over the past decade, we have explored the effects of ghrelin and ghrelin antgaonists on reward behaviours in rodents. We used condition place preference testing to explore the effects on reward from food, alcohol and social reward. We also used this same test to discover whether ghrelin delivery itself is reward/aversive. Motivational tests include operant responding in which animals work increasingly more (by pressing a lever) in order to obtain a food reward. We found that ghrelin does indeed increase reward from most experiences that animals find salient, such as palatable foods, alcohol and even social reward. We also found that activation of the ghrelin system increases food motivated behaviour for sweet treats as well as for regular chow. Many of these behaviours could be driven from the ventral tegmental area and appear to engage the dopamine neurones projecting to the nucleus accumbens. Consistent with its role in hunger, we also found that animals find ghrelin injection to be aversive. Ghrelin's primary physiological role appears to be to enhance food-linked behaviours. At the level of the reward system, these include food reward and food motivation but also reward from natural and artificial rewards.

Symposium 1.2

Ghrelin: Harmonizing Rhythms with Feeding Patterns

Janine L Dovey, Amanda EK Hornsby, Hafsa Kabeer, Timothy Wells
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Temporal patterns are important determinants of hormone action. For example, pulsed ghrelin infusions enhance growth hormone (GH) secretion and skeletal growth, while continuous infusion suppresses GH secretion and promotes fat deposition[1,2]. Since ghrelin secretion is regulated by food intake, we investigated whether feeding patterns regulate ghrelin action. Caloric intake in grazing (GR) and meal-fed (MF) rats was reduced by 20% (P<0.0001 vs *ad libitum* (AL)-fed rats). Reduced weight gain in GR rats (P<0.05) was accompanied by reduced skeletal growth, tibial epiphyseal plate width (EPW) being reduced by 15% (P<0.0001). Although weight gain declined similarly in MF rats, tibial EPW was unaffected. These growth rate effects were reversed in *ghrelin*$^{-/-}$ mice. GR rats exhibited a surge in ghrelin secretion at the end of the light phase, a rapid post-feeding decline and a progressive elevation across the dark phase, with little change in GH secretion. Despite the same initial pre- and post-prandial changes in ghrelin secretion, MF rats showed an additional surge in ghrelin prior to the last dark phase meal, and a 166% increase in GH secretion (P<0.05 vs AL and GR), with two additional pulses of GH per day (P<0.0001 vs AL and GR). In a two diet (low fat (LFD)/high fat (HFD) food choice test, GR rats showed no significant preference for either diet, but MF rats showed a four-fold preference for LFD (P<0.001). This was abolished by maintaining rats on an HFD throughout the study. When maintaining LFD/HFD choice for 6 weeks, MF rats showed no significant preference, whereas GR rats showed a 55% preference for HFD (P<0.001), with an irregular infradian rhythm of HFD selection. Thus, temporal feeding patterns influence biological outcome. Indeed, the contemporary shift from regular meals to less structured feeding may enhance energy-dense food selection and disrupt the relationship between nutrition and growth.

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Symposium 2.1

Rejuvenating the Adult Mammalian Brain – 'Ghrelin' Links Calorie Restriction with Hippocampal Neurogenesis

Amanda K. E. Hornsby[1#], Luke Buntwal[1#], Maria Carla Carisi[1], Vanessa V. Santos[2], Fionnuala Johnston[3], Luke D. Roberts[1], Martina Sassi[1], Romana Stark[2], Alex Reichenbach[2], Sarah H Lockie[2], Mario Siervo[3], Owain Howell[1], Alwena H. Morgan[1], Timothy Wells[4], Zane B. Andrews[2], David J. Burn[3], Jeffrey S. Davies[1*]

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Calorie restriction is known to have beneficial effects on brain function, including improving memory and reducing the incidence of age-related neurodegenerative diseases. During caloric restriction, blood levels of the hormone "ghrelin" are elevated, and in models of neurodegeneration this hormone is protective. In this talk, I will describe recent progress made in characterising the effect of ghrelin on the generation of new adult hippocampal neurones (neurogenesis). Briefly, new neurones are formed from neural stem/progenitor cells (NSPCs) in the adult dentate gyrus (DG) throughout life and contribute to spatial pattern separation memory. Factors that promote neurogenesis may attenuate age-related cognitive decline. Calorie restriction (CR) has been shown to modulate the DG and improve cognitive function, albeit via unknown mechanisms. Previously, we have shown that acyl-ghrelin (AG) increases neurogenesis in the DG and enhances pattern-separation memory (Kent et al.2015). We also show that CR enhances neurogenesis in WT but not in GHSR-null mice, demonstrating that CR induces AHN in a GHSR-dependent manner (Hornsby et al.2016). To determine whether unacylated-ghrelin (UAG), the so-called inactive form of ghrelin, regulates neurogenesis, WT and ghrelin-O-acyl transferase null mice (GOAT-ko) - that lack circulating acyl-ghrelin - were treated with vehicle or UAG for 7-days. Surprisingly, UAG-treated WT mice had reduced proliferating cells ($Ki67^+$), immature neurones (DCX^+) and newborn ($BrdU^+/DCX^+$) immature neurones. GOAT-ko mice had similar reductions in neurogenic markers and impairments in hippocampal-dependent memory that were restored by acyl-ghrelin treatment. To assess the clinical relevance of these findings, we quantified circulating AG:UAG in Parkinson's disease dementia and report a significant reduction compared to both age-matched healthy controls and a cognitively normal PD group. These data identify a novel role for UAG in regulating hippocampal plasticity and suggest that AG and UAG may be opposing regulators of hippocampal neurogenesis and cognition.

Symposium 2.2

Ghrelin Receptor on Metabolic Health and Cognitive Function in Aging

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The aging process is characterized by a progressive decline of metabolic functions, insulin resistance, and cognitive impairment. We and others have reported that Growth Hormone Secretagogue Receptor (GHS-R) plays a crucial role in metabolic regulation. GHS-R is an endogenous G-protein coupled receptor for gut hormone ghrelin, and ghrelin signals the hypothalamus to increase feeding, decrease energy expenditure, and reduce fat utilization. We previously reported that global GHS-R deletion lead to a healthier metabolic profile compared to WT littermates in aging mice. We showed that ablation of GHS-R ameliorates age-associated metabolic impairments, reducing body weight/adiposity, and improving insulin sensitivity. Strikingly, we subsequently found that suppression of GHS-R in neurons completely prevents diet-induced obesity in mice (*Syn1-cre;Ghsr$^{f/f}$*), by increasing energy expenditure. Here, we further assessed metabolic and memory functions of 18-20 months-old *Syn1-cre;Ghsr$^{f/f}$* mice. Similar to young mice, old *Syn1-cre;Ghsr$^{f/f}$* mice showed reduced body weight, adiposity, and improved insulin sensitivity. In addition, we observed reduced inflammation markers in the brain, including hippocampus and cortex regions. Interestedly, old *Syn1-cre;Ghsr$^{f/f}$* mice spent significantly more time exploring the novel object than the familiar object than controls in novel object recognition test, suggesting that old *Syn1-cre;Ghsr$^{f/f}$* mice retained short- and long-term memory better. In addition, old *Syn1-cre;Ghsr$^{f/f}$* mice

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exhibited significantly increased freezing behavior in both contextual and conditioned auditory stimulus in fear conditioning test, suggesting that old *Syn1-cre;Ghsr*$^{f/f}$ mice have better memory retention. Overall, our data suggest that pan-neuronal deletion of GHS-R attenuates aging-associated obesity, insulin resistance, central inflammation, and memory impairment. Calorie restriction (CR) is the only recognized strategy to improve lifespan and health span in all mammals, the metabolic effects of GHS-R suppression in neurons resemble CR-induced metabolic benefits. Thus GHS-R is an exciting novel molecular target for metabolic fitness and cognitive function; application of GHS-R antagonists in neurons has potential to mimic CR in aging.

Oral Communications

OC-01

Catecholamine and Serotonin Signalling in *Kiss1* Neurons is not Required for Reproduction in Mice

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AIM: The functional implication of catecholamines and/or serotonin signalling in the reproductive axis is uncertain, though ex-vivo application of dopamine on brain slices inhibits *GnRH* neuron firing. A population of neurons in anteroventral periventricular nucleus (AVPV) that project to GnRH neurons, including kisspeptin producing neurons (*Kiss1* neurons), co-express catecholamine and serotonin synthesising enzymes including DOPA decarboxylase (DDC) which catalyses the second reaction in both biosynthesis pathways. We hypothesised that catecholamines and serotonin arising from *Kiss1* neurons would have an impact of the reproductive axis. The aim of this work was to study the reproductive function of catecholamines and serotonin specifically produced in *Kiss1* neurons.
METHODS: We selectively knocked out the *Ddc* gene from *Kiss1* cells using CRE-Lox technology by mating heterozygous *Kiss1-Cre* mice with *Ddc* "flox" mice that have loxP sites flanking exon 8 of the *Ddc* gene, a region shared by the two transcript variants. Excision of this exon throws the resultant splice out of frame. Exon deletion was confirmed by PCR. We then examined the effects on basal reproductive hormone levels, latency to puberty onset, and other measures reproductive function.
RESULTS: In both males and females, there was no difference in latency to puberty onset, body and reproductive organ weights, basal gonadotropin levels, and oestrus cyclicity in females and sperm count in males between wildtype and DDC knockout mice. There was also no difference in percentage of animals that sired litter or gave birth, latency to first litter nor litter size.
CONCLUSION: Catecholamine and serotonin signalling specifically in *Kiss1* neurons in mice are not required for puberty onset, fertility and reproduction.
Keywords: Kiss1, DDC, AVPV, Dopamine, Serotonin, Reproduction.

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OC-02

DLG2 Mutations in Hypogonadotropic Hypogonadism

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AIM: The N-Methyl-D-Aspartate Receptors (NMDARs) are ion-channel receptors that respond to the neurotransmitter glutamate. Studies have shown that NMDARs are associated with the timing of sexual maturation in animals. DLG2 encodes a protein of NMDARs. DLG2 gene was recently associated as a candidate gene for hypogonadotropic hypogonadism (HH) which is characterized by impaired pubertal development and infertility. Therefore, we aimed to screen DLG2 sequence variations in hypogonadotropic hypogonadism patients.
METHODS: The coding and flanking regions of the DLG2 were screened for variants in whole exome sequencing data of HH patients. Determined variants were evaluated in the gnomAD and GME databases according to their allele frequencies and only those with a frequency <0.0001 were considered rare (RSV). InterVar was used to classify RSVs by ACMG/AMP criteria. CADD scores were calculated.
RESULTS: The variant screening of DLG2 (HGNC: 2901) showed two missense variants (p.Ala73Gly and p.Ala102Thr) in two patients in the heterozygous and homozygous state, respectively. Identified variants were found extremely rare and not reported in gnomAD. None of these variants was seen in Turkish Peninsula control individuals of the GME database. Both RSVs were categorized as "uncertain significance (VUS)" by InterVar. Both patients were normosmic and have no causal variants in other IHH-related genes.
CONCLUSION: In this study, we detected two RSVs in DLG2 in a large HH cohort. The absence of other variants in the disease-related genes in both cases suggests that the identified DLG2 variants may have deleterious effects in the pubertal process. Our results support the contention the DLG2 mutations that affect NMDAR signaling are associated with HH in human puberty.
Acknowledgement: This work was supported by the Cukurova University Scientific Research Projects (# 11364).
Keywords: Hypogonadotropic Hypogonadism, DLG2, NMDAR.

OC-03

Stress and altered pubertal timing: Is the limbic brain the key?

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AIM: Post-traumatic stress (PTSD) is associated with altered pubertal timing and predator odour exposure (POE) is a classical rodent PTSD model. Hypothalamic kisspeptin, a core component of the gonadotropin-releasing hormone (GnRH) pulse generator regulating the hypothalamus-pituitary-gonadal axis (HPG), is crucial for reproductive function. Kisspeptin neurones in the posterodorsal sub-nucleus of the medial amygdala (MePD) are thought to modulate pubertal timing and anxiety. We test the hypothesis that psychosocial stress, processed by the MePD, is relayed to the GnRH pulse generator to delay puberty.
METHODS: Female mice were exposed to predator odour, 2,4,5-Trimethylthiazole (TMT), for 14 days from postnatal day (pnd) 21 and pubertal onset was monitored. Anxiety was tested using the Elevated Plus Maze (EPM), Light/Dark Box (LDB) and social interaction (SI). The effect of TMT on luteinizing hormone (LH) pulses was measured, on pnd 26 and 29. Additionally, kisspeptin-cre mice were bilaterally injected with hM3Dq-DREADD AAV in the MePD at pnd 13. From pnd 21, CNO was administered via drinking water for 14 days and pubertal onset was monitored.
RESULTS: The TMT-mice showed a significant delay of first estrous (FE; $p<0.001$) without affecting body weight (BW). TMT-mice spent less time exploring the open arm of the EPM and in the light compartment of the LDB, while engaging less in SI during TMT-exposure compared to controls. The TMT group exhibited a reduction in LH pulse frequency on pnd 26 and 29. DREADD

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activation of kisspeptin neurones in the MePD advances FE ($p<0.05$) without affecting BW.
CONCLUSION: Predator odour stress delays puberty, reduces GnRH pulse generator frequency and enhances anxiety-like behaviour, while selective chemogenetic activation of kisspeptin neurones in the MePD advances puberty in female mice.
Funding acknowledgement DI, PHD student funded by the Medical Research Council, which has supported this work.
Keywords: Stress, Puberty, Developmental Biology, Reproduction.

OC-04

Effects of Circadian Rhythm Disruption due to Chronic Constant Light on Ovarian Aging by mTOR Signaling Pathway

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AIM: Period 2 (PER2), a core circadian clock component, functions in steroid production and cell proliferation in granulosa cells. mTOR signaling functions in maintaining ovarian reserve, follicle development, oocyte meiotic maturation. It was shown that there might be a relationship between PER2 and mTORC1. We aimed to show the effect of constant light on ovary morphology, follicle reserve and PER2, mTOR and p-mTOR protein levels in mice ovary.
METHODS: Female Balb-C mice (6-8 week-old) were used. We housed experiment group in constant light and control group in light:dark 12h:12h for 1 week. Open field test was performed. Paraffin-embedded ovaries were cut, and follicle counting was performed on hematoxylin-eosin stained sections. PER2, mTOR and p-mTOR protein levels were evaluated by western blot.
RESULTS: We observed increased number of fecal boli deposits ($p<0.05$) in experiment group but no significant difference in open field test results. We demonstrated that the number of primordial follicles significantly decreased, and the number of pre-antral follicles and atretic follicles significantly increased in experiment group ($p<0.05$). We showed significant decrease in both PER2, m-TOR and p-mTOR protein levels in experiment group ($p<0.05$).
CONCLUSION: We conclude that the constant light housing may affect total PER2 protein level in the ovary. Altered total PER2 protein levels might cause changes in the activity of mTORC1 signaling pathway. Through altered mTORC1 signaling, ovarian morphology and follicle reserve might be disrupted that may cause ovarian aging.
Keywords: Circadian Rhythm, PER2, mTOR Signaling, Ovarian Aging.

OC-05

Effect of Apelin Receptor Signaling on Development of GnRH Producing Hypothalamic Neurons from pluripotent stem cells

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AIM: Hypothalamus is one of the most important brain region which controls hemostasis of body temperature, sleep, hunger and thirst. Loss of hypothalamus function has been correlated with several neurogenic disorders such as Parkinson's disease and Alzheimer's disease. Identification of possible protective and regulatory signaling mechanisms in hypothalamus is of great interest in recent years. Apelin receptor (APLNR) is a G-protein coupled receptor which is activated by Apelin-13 peptide ligand and has various physiological roles during developmental stages and adult body. In this study, regulatory role of APLNR during GnRH producing hypothalamic neurons differentiation was shown by using mouse embryonic stem cells (mESC) as a pluripotent cell source.
METHODS: A derivative of R1 mESC (ATCC, SCRC-1011) line was used to obtain GnRH producing hypothalamic neurons in culture. To identify the role of APLNR on development of GnRH producing cells, APLNRs level was overexpressed and knocked-out by inducible and CRISPR-Cas9 system, respectively. Receptor activation was induced by administration of the peptide ligand Apelin-13 during different stages of differentiation protocol. mESCs were differentiated into EpiSCs by bFGF (12 ng/ml) supplement followed by differentiation into GnRH producing hypothalamus neurons. A cocktail

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of Noggin, EGF, bFGF, FGF8 and DAPT was used to conduct terminal differentiation of hypothalamus neurons for 24 days. GnRH secretion, gene and protein expressions were determined as a marker of GnRH producing hypothalamus neurons. GnRH and MAP-2 immunostaing were used for hypothalamic neuron characterization.
RESULTS: GnRH production was significantly higher after APLNR activation with Apelin-13 and APLNR overexpression in differentiated GnRH hypothalamus neurons. APLNR knockout decreased GnRH production and secretion significantly in vitro. Morphological analysis revealed a similar phenotype for each group indicating the potential role in GnRH production.
CONCLUSION: Our results suggest that APLNR signaling might have a role during development of GnRH producing mature hypothalamus cells in mESCs culture.
Keywords: mESCs, GnRH, Apelin Receptor, Neural Development.

OC-06

Effects of Methylphenidate on GnRH Expression and Release in GT1-7 Cell Line

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AIM: Methylphenidate (MPH) is widely used for treatment of attention deficit hyperactivity disorder in children. Though there are discussions about growth-related effects of its use, the neuroendocrine mechanisms of MPH around puberty has not been studied. This study aims to investigate the role of MPH on gonadotropin-releasing hormone (GnRH) expression and secretion *in vitro*.
METHODS: GT1-7 cells were maintained in DMEM containing 10% FBS and 1% PSN at 37°C under 5% CO_2 humidified atmosphere. Effect of MPH on proliferation and migration at different doses (1 nM-200 µM) for 24 hours was assessed by trypan blue dye exclusion and wound healing (scratch) assays, respectively. To investigate GnRH expression and secretion, cells were cultured in maintenance medium without FBS with or without MPH (200 µM) and samples at different were obtained to investigate GnRH expression by real-time qRT-PCR and GnRH release to medium by ELISA. Normal distribution of the data was analyzed by Shapiro-Wilk normality test. Data from dose-response experiments were either analyzed one-way ANOVA or Kruskal-Wallis followed by Dunnet's multiple comparison or Dunn's multiple comparison tests, respectively. Real-time qPCR and ELISA data was analyzed by Student's t-test.
RESULTS: There were no significant differences between untreated cells and MPH-treated cells in terms of proliferation and migration. *GnRH* expression was significantly reduced at 30 min and 24 hours after MPH treatment compared to control group ($p<0.05$). Interestingly, effect of MPH on *GnRH* expression was in a cyclic-manner. GnRH release was significantly higher than control group at 2 hours after treatment ($p<0.05$), while no differences found between groups at other timepoints.
CONCLUSION: MPH affects both GnRH expression and release independently. Our result suggests puberty-delaying effects of MPH may be partly through GnRH expression and release. Other factors such as kisspeptin and other endocrine parameters should be investigated.
Keywords: Methylphenidate, GnRH, GT1-7 cells, Attention Deficit Disorder, Reproductive Functions.

OC-07

Effects of modulation of certain TRP channels and voltage-gated potassium channel activities on calcitonin gene-related peptide release in isolated rat hemiskull preparations

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AIM: Migraine is one of the most disabling neurological disorders. Cranial meninges are considered to be responsible for generation of migraine-like headaches. Calcitonin gene-related peptide (CGRP) released from trigeminal sensory fibers innervating the meninges plays a key role in pathophysiology of migraine by inducing

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neurogenic inflammation. Transient receptor potential (TRP) and voltage-gated potassium (K+v) channels on meningeal afferents may modulate CGRP release from these nerve terminals by regulating their threshold of excitation. We explored effects and mechanisms of activation of TRPA1, TRPM8, TRPV1, TRPV4, and K+v channels on CGRP release in isolated rat hemiskull preparations.

METHODS: Adult Wistar rats were divided into 9 groups (n=12 hemiskull). Isolated hemiskull preparations from rat heads were prepared. Groups were topically administered TRPV1, TRPA1, TRPM8 and TRPV4 agonists, in artificial-cerebrospinal fluid, including capsaicin (1 µM), cinnamaldehyde (300 µM), menthol (300 µM) and 4αPDD (100 µM), also antagonists including capsazepine (10 µM), HC-030031 (50 µM), AMTB (10 µM) and GSK-2193874 (10 µM), and also K+v channel opener Retigabine (10 µM) for 15 min, alone and together. CGRP concentrations in superfusates collected from the preparations were measured using ELISA. Data were compared by one-way ANOVA.

RESULTS: While agonists of TRPA1, TRPM8, TRPV1 and TRPV4 channels induced significantly CGRP release from meningeal trigeminal afferent terminals ($p<0.01$), these effects were blocked by antagonists including capsazepine, HC-030031, AMTB and GSK-2193874 ($p<0.001$), respectively. Moreover K+v channel opener Retigabine alone reduced basal release of CGRP ($p<0.001$), and also suppressed the agonists-stimulated CGRP release ($p<0.001$), respectively.

CONCLUSION: Our findings suggest that activation of TRPA1, TRPM8, TRPV1 and TRPV4 channels contributes neurogenic inflammation underlying migraine by evoking CGRP release from meningeal trigeminal sensory fibers. Moreover, such TRP channel antagonists and K+v channel openers like retigabine may prevent neurogenic inflammation by modulating CGRP release from trigeminal afferent terminals during nociceptive firing in migraine. Acknowledgement: This study was supported by BAIBU-BAP (Grant-number: 2017.08.02.1212).

Keywords: Migraine, TRP channels, neurogenic inflammation, CGRP, K+v channel opener.

OC-08

Neuropeptide-W Protects Against Stress-Induced Gastric Ulcer in Rats via the Involvement of Capsaicin-Sensitive Vagal Afferent Fibers

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AIM: Neuropeptide-W (NPW), expressed in hypothalamus and peripheral organs, activates hypothalamus-pituitary-adrenal axis, and may have a physiological role in neuroendocrine response to stress. The aim was to evaluate protective effects of NPW on stress-induced gastric injury in rats.

METHODS: Sprague-Dawley male rats were fasted for 24 hours, restrained and immersed in water-bath for 6 h to induce water-immersion restraint stress (WIRS), and NPW (0.1 or 5µg/kg) or saline was injected subcutaneously at 15 minutes before WIRS (n=24), while saline-injected control rats had no WIRS (n=8). For degeneration of vagal afferent fibers (VAD), in some rats (n=24) capsaicin (1%) was applied perivagally under ketamine anesthesia, and 3 weeks later WIRS was induced. Using a laser Doppler, gastric serosal blood flow was monitored under anesthesia. Following cardiac puncture, gastric tissues were removed for macroscopic/microscopic scoring and measurement of myeloperoxidase activity, malondialdehyde and glutathione levels. Gastric NF-κB and cerebral NPW mRNA expressions were detected by RT-PCR. One-way ANOVA was used for statistical analysis.

RESULTS: WIRS decreased mean serosal blood flow, resulted in elevated macroscopic/ microscopic scores compared to control group ($p<0.001$), while myeloperoxidase activity and malondialdehyde level were elevated ($p<0.05$) and antioxidant glutathione was depleted ($p<0.001$). WIRS depressed gastric NF-κB and cerebral NPW mRNA expressions ($p<0.01$). Neither doses of NPW changed gastric NF-κB mRNA. Lower-dose of NPW elevated blood flow ($p<0.001$), abolished WIRS-induced elevations in myeloperoxidase and

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malondialdehyde levels ($p<0.05$). High-dose NPW replenished gastric glutathione and brain NPW expression and reduced scores ($p<0.05$-0.01). Despite that VAD did not alter effects of high-dose NPW, reductions in malondialdehyde and myeloperoxidase, and improvement in blood flow by low-dose NPW were abolished by VAD ($p<0.05$).
CONCLUSION: In stress-induced oxidative gastric injury, NPW provides gastroprotection by improving depressed blood flow and inhibiting ulcer-induced oxidative injury, which involve contribution of vagal afferent fibers.
*Supported by Marmara University Research Fund (SAG-CDRP-141118-0606) and TUBITAK (318S238).
Keywords: Neuropeptide-W, gastric ulcer, water-immersion restraint stress, oxidative damage, inflammation.

OC-09

Mast cell activation alleviates pentylenetetrazole-induced epileptic seizures via serotonin in rats

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AIM: Neuroinflammation plays key role in epilepsy pathogenesis but neuroinflammatory processes underlying the pathogenesis is not well understood. Mast cells are present at neuroendocrine structures like hypothalamus and pituitary, and modulate neuroimmune functions through mediators stored in their granules such as serotonin and histamine. Effects of activated mast cells on epileptogenesis aren't known. We aimed to investigate effects and mechanisms of compound 48/80-stimulated mast cell activation on pentylenetetrazole-induced epileptic seizures in rats.
METHODS: Adult male Wistar rats were divided into 6 groups (n=11). Intraperitoneally, group-1 (control) received saline, groups 2, 3, and 4 received compound 48/80 at a dose of 0.5, 1 and 2 mg/kg, respectively, 30 min prior to 45 mg/kg pentylenetetrazol injection. After observing the significant effect of compound 48/80 at a dose of 2 mg/kg, and to verify mast cell activation-mediated this effect, group-5 received 10 mg/kg mast cell stabilizer cromolyn plus 2 mg/kg compound 48/80 30 min prior to 45 mg/kg pentylenetetrazol. After the verification, to reveal which mast cell-derived mediator mediate this effect, group 6 was given 10 mg/kg serotonin 30 min prior to 45 mg/kg pentylenetetrazol. Behavioral seizures were videotaped for 30 min, then stages of seizures were evaluated by Racine's scale. Data were compared by one-way ANOVA.
RESULTS: 2 mg/kg dose of compound 48/80 produced anticonvulsive effects against pentylenetetrazole-induced epileptic seizures by extending onset-times of myoclonic-jerk and generalized tonic-clonic seizure ($p<0.05$), and by shortening duration of generalized tonic-clonic seizure ($p<0.05$). These effects were reversed by cromolyn ($p<0.05$). In a fashion similar to compound 48/80, serotonin also exhibited anticonvulsive effects against the epileptic seizures ($p<0.05$).
CONCLUSION: Compound 48/80 acts as an anticonvulsant by activating mast cells in a dose dependent manner. Anticonvulsive effects of mast cell activation are mediated by serotonin. Therefore mast cell activation and mast cell-derived serotonin may be a crucial target for treatment of seizures.
Keywords: Mast cells, serotonin, neuroimmune, epileptic seizures.

OC-10

Effects of chronic ghrelin administration on IL-1, IL-6 and TNF- levels in PTZ kindling epilepsy model in rats

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AIM: Epilepsy is a common neurological disorder which is the second leading neurological cause of sudden death. It affects 70 million people worldwide and one-third of these patients do not respond to current drug therapies. However, much of the pathophysiology of epilepsy remains unelucidated. Inflammatory responses have been

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reported to contribute to the pathophysiology of epilepsy. The aim of this study was to determine the possible anti-inflammatory effects of chronic ghrelin administrations in pentyleneterazole (PTZ)-kindling model in rats.

METHODS: For determining the possible anti-inflammatory effects of chronic ghrelin administrations, blood of 60 male Wistar albino rats was collected. The experimental groups that the blood belongs to were as follows: (1) Control group (physiological saline-PS 1 ml/kg+ PS, 1 ml/kg), (2) PS+PTZ group (PS, 1 ml/kg+PTZ, 35 mg/kg), (3) DZP+PTZ group (Diazepam-DZP, 2 mg/kg+PTZ, 35 mg/kg), (4) G20+PTZ group (Ghrelin-G, 20 g/kg+PTZ 35, mg/kg), (5) G40+PTZ group (G, 40 g/kg+PTZ, 35 mg/kg), (6) G80+PTZ group (G, 80 g/kg+PTZ, 35 mg/kg). Serum levels of interleukin-1 beta (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) were determined by ELISA using commercial kits.

RESULTS: Serum levels of IL-1β were significantly increased in PS+PTZ and DZP+PTZ groups compared to the control ($p<0.001$). The serum IL-1β levels of control group were similar with G20+PTZ group. Serum levels of IL-1β were found significantly decreased in G40+PTZ and G80+PTZ groups compared to the control, PS+PTZ and DZP+PTZ groups ($p<0.001$). Serum IL-6 and TNF- levels were increased in PS+PTZ group compared to the control group but this increase was nonsignificant ($p>0.05$). All three pro-inflammatory cytokine levels were significantly lower than the PS+PTZ group in G40+PTZ and G80+PTZ groups ($p<0.001$).

CONCLUSION: In conclusion, we suggest that chronic ghrelin administration has anti-inflammatory effects in PTZ kindling model in rats.

Acknowledgement: This work was supported by Research Fund of the Van Yuzuncu Yil University (Project Number: TDP-2019-7748).

Keywords: Pentylenetetrazole, anti-inflammatory, serum, pro-inflammatory, cytokine.

OC-11

Epileptic seizures are associated with serum apelin levels and oxidation stress in children with epilepsy

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AIM: Epilepsy affects about 65 million people around the world and can be seen in all age groups regardless of gender. The disease has been known for a long time, however, the mechanism is still unknown. In the present study, it was aimed to investigate apelin and oxidative stress levels in children with epilepsy.

METHODS: Children with epilepsy (aged 0-16) who had been diagnosed with epilepsy were included in the study before the start of their treatment. This study included healthy children as the control group (n=28) and children with epilepsy group (n=28). Blood samples were collected and analyzed for apelin, advanced protein oxidation product (AOPP) and DNA damage marker 8-Hydroxy 2-Deoxyguanosine (8-OHdG) levels analyses by ELISA method.

RESULTS: Serum AOPP, apelin, 8-OHdG were found to be similar between the groups. Apelin level in generalized type epilepsy was lower than the control group and the complicated febrile group ($p<0.05$). As the level of apelin increases, the 8-OHdG levels have been determined to decrease ($p=0.48$; $r=-0.266$). It has been found that the number of epilepsy seizures is more common in the generalized type epilepsy ($p<0.05$). While the number of seizures decreased due to the increase in apelin ($p=0.05$; $r=-0.260$), it increased due to the increase in AOPP ($p=0.05$; $r=0.264$). AOPP was higher in focal type epilepsy than control group. As the BMI increases, the AOPP level ($p=0.006$; $r=-0.372$) and the number of seizures ($p=0.01$; $r=-0.501$) have been determined to decrease.

CONCLUSION: Our findings suggest that 1) Apelin reduced the number of seizures by preventing oxidative DNA damage, 2) Increased the number of seizures by the AOPP increase, 3) As the age rises, the number of

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seizures has been determined to lower due to decreased in AOPP level.
Acknowledgement: This study was supported by HUBAP (Project # 17051).
Keywords: Epilepsy, Apelin, Oxidative stress, 8-OHdG.

OC-12

The Role of ERK Pathway in the Neuroprotective Effect of Memantine

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AIM: Memantine, an uncompetitive NMDA receptor antagonist, has been shown to possess neuroprotective properties in different experimental models of neuronal injury. So far, however, the cellular signaling pathways through which memantine exerts its neuroprotective effects have not been fully elucidated. In the present study, we investigated the involvement of the MAPK/ERK pathway in the setting of memantine's neuroprotection in in vivo and in vitro.
METHODS: For in vitro studies, we examined the effects of memantine administered alone or in combination with PD98059, a specific ERK-1/2 kinase inhibitor, on the activation of ERK pathway and cell viability on primary cortical neuron cultures exposed to oxygen and glucose deprivation (OGD) followed by reoxygenation (R) for 6 hours and 18 hours, respectively. In vivo experiments were done by using a cold-induced model of traumatic brain injury (TBI) in mice. Forty-eight hours after the induction of trauma, activation ERK-1/2 signaling was analyzed by Western Blotting. Coronal brain sections taken from mice were stained with cresyl violet and infarct volumes were determined using the Image J (NIH) software.
RESULTS: Memantine (10 μM), when given 10 min before OGD, significantly enhanced the survival of cortical neurons subjected to OGD/R ($p < 0.05$). The treatment of neurons with PD98059 (10 μM), 10 min before OGD, remarkably abolished the neuroprotective effect exerted by memantine in cortical neurons exposed to OGD/R ($p<0.01$). Similar results were obtained in vivo, memantine treatment (20 mg/kg, ip) resulted in a significant reduction of infarct volume 48 hours after trauma ($p<0.05$). However, pre-treatment with PD98059 (1 mM, ic) attenuated the ability of memantine to protect mice against TBI-induced cell death ($p<0.05$).
CONCLUSION: Our results revealed that activation of the ERK pathway mediates memantine's neuroprotective effects at least partially but there might be other important mechanisms of memantine's neuroprotection as well.
Keywords: Memantine, Neuroprotection, PD98059, ERK-1/2, OGD/R, Traumatic Brain Injury (TBI).

OC-13

Autoimmune Hypothalamitis: A Rare Case and Review of the Literature

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AIM: Autoimmune hypothalamitis is described as a rare autoimmune condition in which hypothalamus is infiltrated by lymphocytes and plasma cells.Although several biomarkers cited as related to this rare entity in the literature, none was specific for it.We report a case of isolated autoimmune hypothalamitis with partial empty sella syndrome (ESS), central diabetes insipidus (DI), and hyperprolactinemia and compare our results with data collected so far in the relevant literature.

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METHODS: A 35 years-old woman diagnosed for menopause by a third-party medical center due to her oligomenorrhea presented to our hospital with hyperprolactinemia. Clinically she diagnosed as having central diabetes insipidus and panhypopituitarism. MRI scan revealed an intraventricular mass, which filled and obliterated the infundibular recess on the third ventricle floor. The excised mass was consistent with an inflammatory pseudotumor most likely displaying features of lymphocytic hypothalamitis in microscopic examination. Immunohistochemical analysis ruled out the diagnoses, i.e., plasma cell dyscrasia, Langerhans cell histiocytosis, Erdheim-Chester disease, Rosai-Dorman disease, Ig-G4 related disease.The data extracted from radiological, endocrinological, ophthalmic and pathologic evaluations compared with an extensive literature research on the subject.Immunological investigation revealed the presence of anti-hypothalamus antibodies (AHA), identified by double immunofluorescence and targeting AVP-secreting cells. Anti-pituitary antibodies (APA) also searched were negative.
RESULTS: The etiology of autoimmune hypothalamitis seems to be unresolved so far due to the limited number of cases.Conclusively, three alternative pathogenic mechanisms were proposed, i.e., end result of lymphocytic hypophysitis; an inflammatory process inducing local hypothalamitis; an autoimmune response resulting of hypersensitivity reaction that could be explained either as a deranged lymphoid interruption triggered by infectious agents, and finally an idiopathic hypothalamitis without any explainable underlying mechanism(s).
CONCLUSION: Our case showed us the importance of using immunohistochemistry as well as APA, AHA recognizing vasopressin-secreting cells for differential diagnosis.The challenge in its diagnosis resides in the few numbers of cases reported in the pertinent literature.Its possible underlying mechanisms need further elucidation.
Keywords: autoimmune hypothalamitis, lymphocytic hypothalamitis, isolated hypothalamitis, anti-hypothalamus antibodies (AHA), central diabetes insipidus.

OC-14

Misdiagnosed atypical invasive prolactinoma presenting with anosmia, sphenoid mass and empty sella: a case report

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AIM: Extrasellar extension can be seen in prolactinomas with macroadenomas or giant prolactinomas. We report an invasive prolactinoma with empty sella which is very rare and with unusual presantation of anosmia.
METHODS: A 54 years old woman with high level of prolactin attended to Department of Endocrinology of Ondokuz Mayıs University Medical School in December 2019. In her medical history, she referred to a clinic with headache, nasal stuffiness and anosmia and she had been diagnosed as olfactory neuroblastoma according to her pathology of unilateral sphenoid sinus surgery operation in 2013. As she had residual mass in the sphenoid sinus she took radiotherapy, chemotherapy and cyber knife treatment from 2013 to 2015. Treatments were uneffective so she was followed with stabile mass without treatment for two years till 2017. After unfollowed two years she attended to a clinic with amnesia. Then she referred to first clinic with 4*2 cm mass in the sphenoid sinus and after detection of high prolactin level and evaluation of the pathology the mass is decided to be a prolactinoma. When she attended to our clinic, previous blood tests and radiological images were reevaluated. We thougt that it could be an ectopic prolactinoma with empty sella but we observed a relationship between the mass and the pituitary gland in the first MRIs so we made the diagnosis as invasive prolactinoma with empty sella and we started cabergolin 0,5 mg twice in a week.
RESULTS: Prolactinomas may present with unusual symptoms according to extensions to the structures around the sella turcica. Inferior extension with empty sella may interfere with skull base tumors like olfactory neuroblastoma.
CONCLUSION: Invasive prolactinoma and also invasive pituitary tumors with empty sella must be taken into consideration when evaluating intracranial masses or skull base tumors.
Keywords: Invasive prolactinoma, ectopic prolactinoma, extrasellar extension.

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OC-15

Clinicopathological features of gastroenteropancreatic neuroendocrine neoplasms: a retrospective single-center experience

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AIM: Gastroenteropancreatic neuroendocrine neoplasms (GEP-NENs) are relatively rare tumors. To the best of our knowledge, there is no well-designed and large-scale studies investigating neuroendocrine tumors (NETs) in Turkey. The present study aims to describe the clinical, pathological and survival characteristics of patients with GEP-NENs.
METHODS: Sixty-one patients with GEP-NENs admitted to the Bezmialem Vakif University School of Medicine Hospital between April 2012 and September 2018 were analyzed retrospectively. Kaplan-Meier method was used for survival analysis and log-rank test was performed to analyze the comparisons between groups.
RESULTS: Forty-five of the 61 GEP-NENs patients (73.8%) were neuroendocrine tumor (NET) and 26.2% of patients were neuroendocrine carcinoma (NEC). The mean age of patients was 55.5 years (28-77). The most frequent localization of tumors were stomach (34.4%) and the most common symptom was abdominal pain (27.9%). The rate of distant metastases was 36.1% at diagnosis, 63.9% of the patients were operated. The median follow-up period was 27 months. The rate of three-years overall survival (OS) was 88.5% and five-years OS rate was 86.9%. The variables that can significantly influence the OS rate were high grade (grade 3; p= 0.005), Ki-67 proliferation index (Ki-67> 20%; p= 0.002) and distant metastases (p= 0.018).
CONCLUSION: This study provides an evaluation of the clinicopathological properties of GEP-NENs in the Turkish population. GEP-NENs can develop anywhere in the digestive tract. The prognosis of patients with high grade tumors is poor.
Keywords: Gastroenteropancreatic neuroendocrine tumor, neuroendocrine carcinoma, neuroendocrine neoplasm.

OC-16

Pheochromocytoma/paraganglioma –demographic, clinical, laboratory, and treatment outcomes in a series of 37 patients from a single center

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AIM: Pheochromocytoma and paraganglioma are rare neuroendocrine tumors arising from the adrenal medulla and extra-adrenal ganglia respectively. The hypersecretion of catecholamines from the tumors can be associated with high morbidity and mortality, even when tumors are benign. In this study, we evaluate the demographic, clinical, laboratory features and responsivity level to treatment of the patients diagnosed with pheochromocytoma and paraganglioma in a single center.
METHODS: A total of 37 patients who were diagnosed between 2004-2019 in Department of Endocrinology Ondokuz Mayis University were included in the study.
RESULTS: 51.4% of the patients were pheochromocytoma, 32.4% were paraganglioma. Twenty-one of the patients were female, 16 were male. The mean duration of diagnosis was 4.9 years, the mean age of diagnosis was 43. The most common reason for admission was hypertension and abdominal pain. 22% of the patients were diagnosed incidentally. Although 59% of the patients had hypertension, 21% had a history of hypertensive crisis. At the time of diagnosis, 24-hour urine catecholamine results were reached of 34 patients, and 18 of these had more than 2-fold elevations in catecholamine levels. The highest catecholamine was normetanephrine. Specific findings were seen in 34 patients on radiological imaging. The first treatment option was surgery. While 1 patient with head and neck paraganglioma was kept under close follow-up, 3 patient could not operated due to surgery technical difficulties and comorbidites.

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CONCLUSION: The female to male ratio, mean age at the time of diagnosis, the rate of functionality and radiological features were found to be compatible with the literature. 62% of patients were diagnosed in the last 5 years and it was associated with the development and widespread use of imaging methods. Despite radiological and biochemical diagnosed easily, broad and non-specific complaints are causing delayed and missed diagnoses. The frequencies found in the autopsy series support that these patients are not adequately diagnosed.
Keywords: Pheochromocytoma, paraganglioma, hypertension.

OC-17

Diosgenin Exhibits Antitumor Effect in SH-SY5Y Neuroblastoma Cell Line by Inducing Apoptotic Gene Expressions and Reducing Cell Cycle Related Gene Expressions

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AIM: Neuroblastoma (NB), an embryonal cancer of the sympathetic nervous system, is the most common cancer diagnosed during the first year of life. Diosgenin (DSG) is a naturally occurring steroidal sapogenin and is one of the major bioactive compounds found in dietary fenugreek (Trigonella foenum-graecum) seeds. The aim of this study is to investigate the anticancer effect of DSG on SH-SY5Y human neuroblastoma cells.
METHODS: 5-10-20-40-60-120-240 µM concentrations of DSG were prepared for cytotoxicity assay. SH-SY5Y cells were seeded to 96-well cell culture plates (10.000/well). After 24 h incubation, cells were treated with DSG concentrations and incubated for 24, 48 and 72 hours. XTT (2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide) method was used for determining the cytotoxic effects of DSG. Formazan crystals formation were measured at 450 nm wavelength with a microplate reader and IC50 dose of DSG was calculated. Cells were treated with IC50 dose of DSG and RNA isolation was performed by Trizol protocol. CASP-3, CASP-8, CASP-9, CASP-10, BCL-2, BCL-XL, BID, PUMA, NOXA, APAF-1, FADD, TRADD, DR4, DR5, P53, TNF, P21, CYCLIN E, CYCLIN B, CYCLIN D2, CDK4, CDK6, RB genes expression levels were analyzed through SYBR Green method in comparison to GAPDH housekeeping gene.
RESULTS: IC50 dose of SH-SY5Y was calculated as 45 µM at 24th hour. CASP-3, CASP-8, CASP-10, BCL-XL, NOXA, FADD, TRADD, P53 gene expressions were significantly increased and BCL-2, CYCLIN E, CYCLIN B, CYCLIN D2, CDK4, CDK6 gene expressions were significantly decreased in the dose group compared with control group.
CONCLUSION: Our data demonstrate that DSG treatment decreased SH-SY5Y cell viability in a time- and dose-dependent manner. It is noteworthy that DSG induced apoptosis related gene expressions and reduced cell cycle related gene expressions. DSG may be a candidate agent for neuroblastoma but detailed in vitro and in vivo studies are needed for determining its molecular mechanism.
Keywords: Diosgenin, Neuroblastoma, Apoptosis.

OC-18

Environmental stress promotes lipid droplet accumulation in rat brain astrocytes

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AIM: Astrocytes, a subtype of neuroglial cells in the central nervous system (CNS), are involved in the regulation of CNS energy metabolism. Opposed to glucose metabolism, mechanisms underlying the regulation of lipid metabolism in astrocytes are understudied. During CNS pathologies lipid droplets (LDs), lipid storage organelles, storing free fatty acids (FFAs) and cholesterol, tend to accumulate in glial cells and not neurons. However, the mechanisms leading to LD accumulation in glial cells during CNS pathologies, are largely unknown. Here we studied, whether environmental stressors

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related to CNS pathologies affect accumulation and mobility of LDs in astrocytes.
METHODS: Astrocytes in culture and organotypic brain tissue slices were exposed for 24 h to nutrient deprivation (replacement of growth medium with 10 or 0 mM glucose in extracellular solution), excess of FFAs (300 μM oleic acid) or L-lactate (20 mM), hypoxia (1% O2) and adrenergic agonists/antagonists to determine whether noradrenaline, the major stress-related CNS neuromodulator, affects LD content in astrocytes. LDs in astrocytes were labeled with fluorescent markers (Nile Red, BODIPY493/503) and the mobility and the content of LDs evaluated by confocal microscopy.
RESULTS: Nutrient stress (nutrient deprivation, surplus of FFAs or L-lactate), oxidative stress (hypoxia) and adrenergic cAMP signaling increased the formation of LDs in isolated and brain tissue astrocytes over 2-fold. LD mobility in astrocytes was reduced under nutrient deprivation.
CONCLUSION: Our results show that LD mobility is decreased, while LD content is increased in isolated and brain tissue astrocytes by environmental stressors, indicating LD accumulation in astrocytes, as observed in CNS pathologies. During stress FFAs from LDs can be utilized in mitochondrial β-oxidation as an alternative energy source. Moreover, LDs may protect cells against the FFA lipotoxicity, thus increasing viability of neural cells.
Acknowledgement: This study was supported by the Slovenian Research Agency.
Keywords: Lipid droplets, Stress, Astrocytes, Confocal microscopy.

OC-19

Paraventricular Hypothalamic Nucleus (PVN) Glucosensitive Neurons Stimulate Food Intake in 18 Hours Food Deprived Male Rats

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AIM: By using paraventricular nucleus (PVN) doubled-labeled with immunohistochemistry, we showed the existence of glucosensing neurons in PVN. Modulation of neuronal activity by glucose is pivotal in coordinating energy balance and behavior. Therefore, we hypothesized that the PVN glucosensing neurons may be involved in the control of food intake and energy balance.
METHODS: Male wistar rats (220-250 g) implanted with guide cannula directed to the PVN based on paxinos coordination, lateral: +0.4 mm from midline; dorsoventral: 7mm from skull surface; anteroposterior:-1.8 mm from the bregma. Glucose (0.3, 0.8, 1.5 μg) and saline (0.3 μl) were microinjected into the PVN and food intake was measured over 1 h. Feeding trials normally occurred from Saturday to Wednesday between 9:00 and 10:00 h. All drugs were prepared freshly just before test and they were administered in distilled water. Plasma glucose was measured by ELISA method.
RESULTS: Intra-paraventricular injection of glucose (0.3 μg, 0.8 μg, 1.5 μg) increased feeding in a dose-dependent manner. The amount of glucose-induced food intake reached statistical significance at doses of 0.8 and 1.5μg ($P < 0.001$) after 1 h. The plasma glucose concentration reaches the maximum value after PVN-microinjected glucose.
CONCLUSION: The present study suggests that PVN-microinjected glucose stimulates food intake through, at least in part, glucosensing neurons.
Keywords: Hypothalamic paraventricular nucleus, Food intake, Glucose, Glucosensitive neurons.

OC-20

The Alterations in Central and Peripheral Apelin in Maternal Diet-Induced Obesity

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AIM: In modern societies, increasing prevalence of obesity and obesity-related diseases yield a substantial consideration for restrictive diet approaches. By contrast, exposure to maternal high fat diet (HFD) during perinatal period is known to predispose to obesity and obesity-associated comorbidities. Apelin has a widespread distribution in central nervous system (CNS) and peripheral tissues including white adipose tissue. Apelin has been

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demonstrated to influence energy expenditure and metabolism, moreover, higher serum apelin levels were observed in obese individuals. The present study was designed to investigate the perinatal HFD-induced alterations in apelin and obesity phenotypes.
METHODS: Pregnant Wistar rats were fed with a control diet or HFD (13.5% or 60% energy by fat, respectively) from embryonic day-14 to postnatal day-21. Following sexing, all male pups consumed standard rat chow till the experiments. For detection of apelin, serum and cerebrospinal fluid (CSF) were collected at 12 week-old age. Body weight and food intake were monitored throughout the experimental period; whereas, epididymal adipose tissue was harvested to evaluate visceral adiposity.
RESULTS: Compared to control, rats exposed to perinatal HFD consumed greater amount of chow (0.10±0g vs 0.01±0g, $p<0.05$) throughout the experimental period, while body weight (321.2±3.97g vs 235.5±9.35g, $p<0.05$) and visceral adiposity were significantly higher. Elevated apelin levels were detected both in serum and CSF in perinatal HFD rats.
CONCLUSION: Although the pups solely consumed a regular diet, they exhibited a remarkable phenotypic switch to obesity along with increased apelin production in circulation and microenvironment of CNS. Apelin appears to play a role in obesity both through endocrine and neuronal signaling. Although it is not entirely addressed in this study, APJ receptor signaling may provide a novel target for treatment of diet-induced obesity.
Keywords: Perinatal High Fat Diet, Obesity, Apelin, Cerebrospinal Fluid, Serum.

OC-21

Neurokinin 3 Receptor Effects on Cognitive Behaviours in a Rat Model of Alzheimer's Disease

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AIM: Alzheimer's disease (AD) is known as a form of progressive and irreversible dementia. Cholinergic neurotransmission is commonly affected in AD. Neurokinin B (NKB) is a hormone belonging to tachykinins family. Neurokinin 3 receptor (NK3R), the NKB receptor, is involved in learning and memory related processes. Activation of NK3R is known to facilitate the release of many neurotransmitters such as acetylcholine (ACh). Aim of this study was to investigate effects of NK3R agonist senktide on cognitive functions and neurobehavioral mechanisms in experimental model of AD.
METHODS: 50 adult male Wistar albino rats were obtained; 1) Control 2) AD 3) Control+NK3R agonist 4) AD+NK3R agonist (ADS) 5) AD+NK3R agonist+antagonist groups. Experimental AD model was established by applying amyloid beta1-42 (2,2 nmol/10µl) intracerebroventricularly. Following NK3R agonist (0,2 mg/kg)and antagonist (6 mg/kg) injections, open field (OF), Morris water maze (MWM) and new object recognition test (NORT) were applied for behavior and learning parameters. Analysis of cholinergic mechanisms was performed from hippocampus and cortex tissues by ELISA method. SAS University Edition 9.4 program was used for statistical analysis.
RESULTS: Group-time effect was statistically significant in OF test ($p<0.05$). There was a statistically significant difference between groups at test stage of MWM ($p<0.05$). The distinction and recognition indices were evaluated in short and long term NORT. There was no significant difference in NORT between the groups. AChE and ChAT levels were determined by ELISA in hippocampus and cortex tissues. There was a statistically significant difference between the groups by AChE and ChAT levels ($p<0.05$).
CONCLUSION: NK3R agonists were found to be effective in improving cognitive functions in rats with AD pathology. It has been observed that positive effects on learning and memory performances can be mediated by cholinergic mechanisms. NK3R should be further investigated for possible pharmacological applications in AD and other cognitive disorders.
Keywords: Alzheimer, neurokinin 3 receptor, memory.

OC-22

Selection of Housekeeping Genes for Rat Hippocampus Expression Studies: Alzheimer's Disease Model

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AIM: Quantitative real time polymerase chain reaction (qPCR) analysis are often used for molecular level understanding of pathophysiology of Alzheimer's Disease (AD). Housekeeping genes (HKGs) are widely used as an internal control and for normalization of qPCR data. Previous literature reported that different experimental procedures can effect expression of HGKs. If so, these changes may cause incorrect normalization and evaluation of the results. Objective of this study was to select appropriate HKGs to be used in normalization for gene expression of rat hippocampus in AD model with or without a neurokinin B receptor agonist (senktide) treatment.
METHODS: Experimental model constituted in 24 male adult rats which were divided into four groups including control, AD, control+0,2 mg/kg senktide and AD+0,2 mg/kg senktide. Drug infusions were subcutaneously performed for eight days. Hippocampal tissues were used for total RNA isolation and cDNA synthesis. mRNA level expressions of 9 HKGs were evaluated using qPCR. Descriptive statistical and average expression stability values (M) were separately calculated for each experimental groups and in general using BestKeeper, geNorm and NormFinder software.
RESULTS: Similar M values were calculated using geNorm and NormFinder algorithms in general. SDHA was the most reliable reference gene with stability value of 0,067 (geNORM) and 0,013 (NormFinder). NormFinder also suggested that PGK1 and CycA were the best combination of two genes with M=0,011 value.
CONCLUSION: RLP13A and SDHA were found to be the most stable HKGs for normalization of expression analysis using rat hippocampus tissues in Alzheimer Disease (AD) model.
Keywords: Alzheimer's disease; housekeeping genes, qPCR, rat, hippocampus.

OC-23

Systemic Sodium Hydrosulfide Causes Neuronal Degeneration in a Dose-Dependent Manner in Cerebrum but not Through the Oxidative Stress Pathway: An Experimental Study

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AIM: Hydrogen sulfide (H2S) is a gaseous endogenous neurotransmitter. Recent studies showed low amounts of H2S upregulate the endogenous antioxidant system and exert additive beneficial effects together with antioxidant agents such as N-acetylcysteine, glutathione, and superoxide dismutase (SOD). Sodium hydrosulfide (NaHS) is the product of the half-neutralization of H2S. In this study, the systemic NaHS administration on the cerebral cortex and cerebellum tissues in rats were investigated.
METHODS: Twenty-four Sprague Dawley male rats were divided into 3 groups by simple randomization: Control Group (n=8), low dose NaHS intravenously applied group (LNaHS: 10 μmol/kg NaHS single-dose, n=8), high dose of NaHS administered group (HNaHS: 30 μmol/kg NaHS single-dose, n=8). For biochemical evaluation, malondialdehyde (MDA), reduced glutathione (GSH), SOD, catalase (CAT) and glutathione peroxidase (GPX) were measured in the cerebral cortex and cerebellum tissues and other half of the same tissue sections were also evaluated histopathologically by hematoxylin-eosin staining. According to the normality results, one-way ANOVA test was used for the statistical analysis. If needed, multiple comparisons were carried out by Tamhane's test. A p-value less than 0.05 was considered statistically significant. Data were presented as mean (95% CI).
RESULTS: MDA, GSH, SOD and CAT levels determined in the cerebral cortex and cerebellum tissues were not found as statistically different among the groups ($p>0.05$). However, GPX level was significantly

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decreased in the cerebral cortex tissue in the LNaHS group (7.5 U/g (95% CI [5.07-9.93]) when compared to the control group level (21.4 U/g (95% CI [15.5, 27.3]) ($p<0.05$). When evaluated histopathologically, the neuronal damage scoring at the cerebrum in the HNaHS group was statistically significantly higher than the control ($p<0.01$) and LNaHS ($p<0.05$) groups.
CONCLUSION: Further studies are needed to better understand the molecular mechanism(s) of oxidative-stress independent neuronal damage caused by HNaHS.
Keywords: Hydrogen sulfide, neuronal degeneration, neurotoxicity, oxidative stress, sodium hydrosulfide.

OC-24

Effects of Transcranial Direct Current Stimulation on Learning and Memory Changes after Experimental Cerebral Ischemia

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AIM: Cerebral ischemia occurs as a result of obstruction of some or all of the arteries feeding the brain, resulting in significant impairments in both motor and cognitive functions. In recent years, direct current stimulation (DCS), a noninvasive treatment method, is used frequently in the treatment of learning and memory disorders. In the present study, we investigated ischemia/reperfusion (IR)-induced changes in learning and memory in male rats, and the effects of treatment with tDCS were elucidated.
METHODS: Sixty male Wistar rats weighing 300 g were divided into 4 groups as sham, IR, DCS and IR+DCS. The IR model was created by MCA's 90-minute occlusion. DCS treatment was applied 30 minutes a day for 7 days after IR. Open field test was used to evaluate motor functions, Novel Object Recognition (NOR) and Y Maze tests were used to evaluate learning and memory. Ischemic injury evaluated with triphenyltetrazolium chloride (TTC).
RESULTS: Compared to the sham group, there was a significant decrease in both motor and cognitive functions in the IR group ($p<0.05$). When the motor functions after DCS treatment were evaluated, there was a statistically significant increase in the distance and velocity parameters taken in the IR + DCS group compared to the IR group. The learning and memory results were similar to the motor function results, and increased in the IR + DCS group in the NOR and Y maze tests. It has been determined that DCS treatment reduces ischemic damage and that the TTC result has less damage in the IR + DCS group than in the IR group.
CONCLUSION: In summary, noninvasive DCS treatment applied after ischemia/reperfusion was found to play a role in improving both motor and cognitive functions.
Our project was supported by Coordination Unit of Scientific Research Projects of Akdeniz University (Project number: TDK-2018-3712).
Keywords: Cerebral Ischemia, Transcranial Direct Current Stimulation, Learning and Memory.

OC-25

Hippocampal and Hypothalamic FBN1 Expression in an Experimental Opioid Addiction Rat Model

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AIM: FBN1 encodes a profibrillin and C-terminal cleavage product of this proprotein result in a fasting-induced protein hormone named Asprosin. FBN1 has critical roles in the formation of elastic fibers in connective tissues. Asprosin activates G protein-cAMP-PKA pathway and rapidly elevates serum glucose level. Asprosin can also cross blood-brain barrier and regulates hypothalamic neurons. In this study, hippocampal and hypothalamic FBN1 expression levels were investigated in an experimental opioid addiction rat model.
METHODS: A total of 18 rats were randomly divided into three equal groups including control (C), morphine dependent (M) and morphine dependent+ naloxone (M+N). Experimental dependence was constituted via applying 10 mg/kg/day subcutaneous morphine injection for 7 days. M+N group received intraperitoneal 1 mg/kg naloxone 1.5 hour after morphine injections. All animals were evaluated for morphine withdrawal symptoms and compared with the other groups. Hippocampus and hypothalamus tissues were dissected and mRNA level expression of FBN1 was evaluated by using quantitative RT-PCR.

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RESULTS: Steady state FBN1 expressions were determined in both hippocampus and hypothalamus. Morphine treatment significantly decreased FBN1 expression in hippocampus ($p<0.01$). An insignificant downregulation was also observed in hypothalamus of morphine group. Naloxone treatment tends to upregulated FBN1 expression in both hippocampus and hypothalamus. However, a significant increase was determined in M+N group compared to hypothalamus of C ($p<0.05$) and M ($p<0.01$) groups.
CONCLUSION: These findings suggest that asprosin and/or other possible active peptides derived from FBN1 transcript may have modulatory roles in hippocampal formation. Additionally, in morphine dependent rat, increased FBN1 levels in M+N group comparing to both control and morphine groups indicated that there is an important opioid tonic inhibition on FBN1 expression in hypothalamus.
Key words: Hypothalamus, hippocampus, opioid addiction, FBN1, asprosin.

OC-26

Effects of Melatonin on Brain Damage and Synaptic Transmission in Septic Rats

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AIM: The oxidant/anti-oxidant balance system is disrupted in sepsis, an important forms the basis of the defense mechanism in the cell, caused by the host's irregular response against to bacterial infection. Septic encephalopathy is one of the most common cases of sepsis. Melatonin is a strong endogenous anti-oxidant hormone which secreted by the pineal gland. Lipopolysaccharide (LPS) is a gram negative bacteria's cell wall component. It is used to experimentally creating sepsis model. We aimed to investigate the effects of melatonin (ML) on neuronal damage and synaptic transmission on brain tissue of rats with LPS-induced sepsis.
METHODS: Adult *Wistar albino* rats was divided into 4 groups as; Control, LPS (10 mg / kg i.p.), ML (10 mg / kg i.p. x3), ML+LPS (ML+LPS). Rats were decapitated 6 hours after first LPS injection and brain tissues were taken in 10% formaldehyde. Immunofluoresce method was evaluated immunoreactivities with using NeuN for live neuron, S100- ß for degenerated neuron, and synaptophisin antibodies for synaptic vesicles. Statistical analysis was performed with one-way ANOVA and post-hoc Tukey.
RESULTS: NeuN staining it means counts of living neurons were significantly decreased in LPS group compared to control groups ($p<0.01$), also decreased in both of melatonin and Melatonin+LPS groups ($p<0.05$). S100-ß staining it means counts of degenerated neurons were increased in LPS group compared to control groups ($p<0.01$), also decreased in both of ML and ML+LPS groups ($p<0.05$). Synaptophysin involvement decreased in LPS group compared to other groups ($p<0.05$).
CONCLUSION: We observed that ML administration improved the counts of living neurons and prevented damage to neurons and did not cause any loss in synaptic vesicle proteins.
Acknowledgement: This study was supported by I.U. BAP, project no: 25047.
Keywords: Sepsis, Melatonin, LPS, immunofluorescence.

OC-27

Investigation of the Effects of Pregnenolone Against Oxidative Stress Induced by Anticancer Drug Paclitaxel in Rats

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AIM: Anticancer therapy with employing paclitaxel (PTX) causes serious side-effects, e.g. neurotoxicity and oxidative stress. PTX accumulates in neuronal cells, which are sensitive to oxidative insults, and ROS have been involve in many neurodegenerative processes, including Alzheimer's disease. Pregnenolone, considered

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as a neurosteroid, is an important endogenous modulator of of several brain-related functions. The aim of this study was to investigate the effects of pregnenolone an in vivo model of paclitaxel induced oxidative stress.
METHODS: Rats were randomly divided into four groups, 6 rats each. Animals in group I served as normal group and received the vehicle, whereas those in group II, III and IV were injected with PTX (8 mg/kg, ip). Two weeks after the injection of PTX, rats in group III (4 mg/kg/day, p.o) and IV received pregnenolone (8 mg/kg/day, p.o) over a period of 2 weeks. Behavioral assay was evaluated by locomotor activity test at the different time points. Serum level of IL-1β was measured to evaluated inflammation, while oxidative damage was assessed by total antioxidant status (TAS) and total oxidant status (TOS).
RESULTS: There was no significant difference between baseline points of locomotor activity in the rats receiving paclitaxel and control groups. After treatment, significant decreases in total distance, horizontal, vertical and ambulatory activity, stereotypy movements, and an increase in resting time were observed in the group II compared with the groups I, III and IV($p<0.05$). The level of IL-1β in groups III and IV were significantly lower than group I.TOS activity in group II were significantly higher than that of the grous I, III and IV whereas TAS activity in group II were remarkably lower than that of the other groups ($p<0.05$). IL-1β, TAS and TOS levels showed no significant difference between the grous I, III and IV.
CONCLUSION: These results indicate that pregnenolone provides improvement toward reducing PTX-induced oxidative stress and inflammation.
Keywords: Pregnenolone, oxidative stress, inflammation, paclitaxel.

OC-28

Investigation of the Effects of Pregnenolone on Behavioral Tests Against Cisplatin-Induced Toxicity in Rats

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AIM: The neurosteroid pregnenolone is potent endogenous neuromodulator and has various actions in the central nervous system. Although pregnenolone has particular roles in brain functions, including effects on cognition, emotion, motivation and motor skill, the exact mechanism of pregnenolone action is not clear. The aim of this study was to investigate behavioral effects of pregnenolone using an animal model of cisplatin toxicity.
METHODS: Rats were randomly allocated into 4 groups. The created groups were control (C), cisplatin (CP), CP+4 mg/kg pregnenolone, CP+8 mg/kg pregnenolone. In the rats of other groups rather than C, single dose cisplatin (7,5 mg/kg) were administered intraperitoneal (i.p). Pregnenolone was given orally for consecutive 14 days prior to the treatment of cisplatin at 15th day. For the behavioral analysis, spontaneous locomotor activity of all animals was tested at 72 h after cisplatin injection. For the statistical analyses of the locomotor activity we calculated the means for 10 min periods for the time-line profiles. $P<0.05$ was considered to be statistically significant.
RESULTS: Several behavioral parameters were determined in the locomotor activity test. Compared the other groups, CP group demonstrated significant decrease of ambulatory, vertical and horizontal activity, but increasing resting time ($p<0.05$).
CONCLUSION: The results demonstrate that CP, given at a toxic dose, caused several adverse behavioral alterations in rats. It has been determined that pregnenolone has a positive effect on behavioral parameters in rats with CP toxicity. However, further studies are needed to investigate the mechanism of action of pregnenolone on CP toxicity.
Keywords: pregnenolone, cisplatin toxicity, behavioral tests.

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OC-29

Pituitary Adenylate-Cyclase-Activating Polypeptide Mediates Fear Responses in Ventrolateral Periaqueductal Gray

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AIM: The periaqueductal gray (PAG) region modulates species-specific defensive reactions. Given the PAG's cell complexity, dissecting specific mechanisms responsible for defensive reactions is elusive. We aim to investigate whether Pituitary adenylate-cyclase-activating polypeptide (PACAP) modulates fear behavior.
METHODS: PACAP-EGFP mice were used for immunohistochemistry (IHC) analysis to characterize PACAP-positive cells in PAG. Optogenetic behavioral experiments were conducted with PACAP: Cre mice. On day1, both ChR2 and EYFP reporter animals received five light stimulation presentations. On day 2, animals were placed to the same behavior chamber and received five presentations of foot-shock (0.9 mA shock intensity, 1 sec duration). On day 3, mice received four presentations of white noise at 75 dB with or without light stimulation. Further behavior experiments were obtained from PAC1 *flox/flox* mice. Both ChR2 and EYFP reporter mice received four presentation of shock paired with white noise on day 1. On day 2, white noise without shock is presented to measure freezing and darting activity. Animals were transcardially perfused for further analysis.
RESULTS: Data obtained from IHC analysis showed that PACAP cells are sparse in dorsomedial and dorsolateral PAG, whereas there is a moderate amount of PACAP cells mainly located in ventrolateral PAG. Further, those PACAP positive cells also show *c-fos* activity during fear conditioning, suggesting they may participate in the stress response. A majority of these PACAP cells overlap with VGAT positive neurons, indicating they are inhibitory neurons. Behavioral data showed conditional deletion of the PAC1 receptors enhanced the activity burst and attenuated the freezing response. Further, light stimulation increased the freezing response, but it did not change baseline activity for either group.
CONCLUSION: PACAP neurons-overlapping with VGAT in vlPAG modulate fear behavior and show *c-fos* activity. Activating PACAP cells induces fear sensitization through the shock, whereas deleting PAC1 receptors shifts the response from fear (freezing) to panic (activity burst and darting).
Acknowledgement: This study was supported by National Institute of Mental Health. RO1-MH62122 to MSF.
Keywords: PACAP, PAC1 receptor, Periaqueductal gray, Fear conditioning.

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OC-30

Non-invasive Vagal Nerve Stimulation Shifts Liking to Lower Fat Stimuli and Increases Striatal Brain Response in Humans

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AIM: The effectiveness of bariatric surgery, which disrupts vagal innervation to the gastrointestinal tract, suggests a role for gut-brain signaling in obesity. The invasive nature of this intervention results in significant adverse effects and is associated with higher morbidity and mortality rates. We propose to use a non-invasive approach to electrically stimulate the auricular branch of the vagus nerve (ABVN), which supplies the skin of the concha in the ear. ABVN stimulation (ABVNS) activates the nucleus of the solitary tract (NTS) similarly to cervical vagal nerve stimulation. In diet-induced obese rats ABVNS has been successfully used to prevent weight gain.
METHODS: We used ABVNS during various food perception tasks in participants with healthy weight (n=10, 6f, 27 yrs old +/-4). Participants sampled puddings with

varying fat content and made liking ratings on a line scale during ABVNS and sham stimulation (control task). We also measured neural responses with functional magnetic resonance imaging when participants (n=4, 2f, 27 yrs old +/-3) tasted low fat milkshakes during ABVNS and sham.
RESULTS: ABVNS shifted liking towards lower fat stimuli compared to sham stimulation. We validated activation of NTS by ABVNS vs sham. We also observed increased response to milkshake (fat % similar to low fat solutions in the food perception tasks) vs tasteless in hypothalamus and dorsal striatum.
CONCLUSION: This corroborates animal studies pairing vagal stimulation with choice for solutions containing different fat concentration, in which vagal stimulation increased both preference for low calorie fats, and potentiated striatal dopamine release in response to low fat. As ABVNS is entirely non-invasive this may be a promising intervention that can be used at a much early stage of obesity than bariatric surgery or even for prevention of overweight individuals tracking to obese status.
Keywords: Food perception, obesity, vagus nerve stimulation.

OC-31

Injection of Ghrelin into Hippocampal Area CA1 and Aerobic Exercise Act to Enhance Learning and Passive Avoidance Memory in Adult Male Rats

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AIM: Ghrelin plays important role in neurological effects such as reward, learning, memory and cognition. Also, scientific evidence has shown that physical activity improves cognitive health across the human lifespan. Exercise mostly exerts its effects on cognition by affecting on hormones related to the management of energy metabolism and synaptic plasticity. Therefore, in this study, we aimed to evaluate the effects of ghrelin and aerobic exercises on passive avoidance memory in rats.
METHODS: Experiments were performed on 64 male Wistar rats, with an initial weight of 250 ± 20 g, 3-4 months old in eight groups. Ghrelin concentration which injected to rats was (3 nM). Exercise groups performed aerobic exercise trainning for 8 weeks. Control groups received same volume of saline. After the ending of treatments, animals set on passive avoidance training by shuttle box apparatus and their memory was examined after 15 min, 24 hr, 48 hr and 10 days. SPSS software was used to analysis the data and $P < 0.05$ was considered significant.
RESULTS: Our findings showed that combined effects of ghrelin and 8 weeks aerobic exercise increased step-through latency (STL) and decreased time spent in the dark compartment (TDC) in acquisition ($P<0.001$), consolidation ($P<0.01$) and retrieval ($P<0.05$) of passive avoidance memory compared to their counterparts.
CONCLUSION: The results of this study indicated that the combined effect of ghrelin and aerobic exercise improves acquisition, consolidation and retrieval of passive avoidance memory.
Keywords: Aerobic exercise, ghrelin, learning.

OC-32

Infusion of Intracerebroventricular Irisin to Obese Male Rats Increases Secretion of Reproductive Hormones

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AIM: Obesity is a chronic disease characterized by excess weight gain. Studies show that obesity is an important reason for infertility. The presence of the irisin in the tissues involved in the reproductive axis, such as the hypothalamus and testis, has been determined. The presence of irisin in these tissues suggests that the peptide can play roles on male reproductive hormones. This study was conducted to investigate the effects of intracerebroventricular irisin infusion on reproductive hormones in rats with an experimental obesity model.
METHODS: Forty male Wistar rats were used in the study. They were divided into 4 groups with their body weights close to each other (n=40). 21 day old rats were

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fed with high fat diet for 12 weeks. The obesity model was confirmed by the Lee index. The rats in the test groups were infused to the lateral ventricles using osmotic mini pumps in a volume of 5µl/hour for 14 days (artificial Cerebro Spinal Fluid in the sham group, 10 and 100 nM irisin in the administration groups). The animals were decapitated at the end of the experiments, and blood samples were collected. Serum FSH, LH and testosterone levels were determined using the ELISA method.
RESULTS: Infusion of irisin caused increases in serum FSH, LH and testosterone levels in rats ($p<0.05$). It was determined that the increase in serum FSH level was similar in concentrations of 10 and 100 nM irisin. The increase in serum LH and testosterone levels were determined to be dose dependent ($p<0.05$).
CONCLUSION: The present study shows that obesity increases the secretion of reproductive hormones in obesity and can play important physiological roles in reproductive functions. These results suggest that irisin might have important place in the treatment of reproductive disorders such as obesity-related infertility.
This study was supported by TUBITAK (Project# 214S205).
Keywords: Irisin, obesity, FSH, LH, testosterone.

OC-33

Effect of Bee Venom on Behavior and Its Relationship with Leptin Levels

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AIM: Anxiety is the feeling of worry, even fear, against the danger that a person perceives from inside or outside, caused by different situations. The leptin hormone released from adipose tissue is known for function in the control of metabolism. It has been shown in recent studies that leptin plays a role in the regulation of mood disorders. However, the available data explaining this is limited and contradictory. Bee venom (BV) has anti-inflammatory, anti-rheumatoid and analgesiceffects. It has been shown that BV prevent the migration of cancer cells and cell differentiation due to obesity. The aim of this study was to evaluate the dose-dependent effect of BV on behavioral functions in rats by comparing leptin levels in prefrontal cortex, hypothalamus and amygdala tissues.
METHODS: Adult male Spraque-Dawley rats were used in the experiments, rats divided into 3 groups (n=7 / group) as control, 0.1mg/kgBV and 0.5mg/kg BV. Rats were injected with BV subcutaneously for 15 days. On the 15th day, the animals were administered behavioral open field test (OFT), elevated plus maze test (EPM) and forced swimming test (FST). The rats were sacrificed on the 16th day, and brain regions were taken. Leptin levels were evaluated in tissues by Elisa method.
RESULTS: In the OFT, it was observed that the total distance and speed in 0.1mg/kg BV group increased compared to other groups($p<0,001$).In the EPM, 0.1mg/kg BV group remained in the open arm for a significantly longer time compared to other groups ($p<0,001$).In the FST,0.5mg/kg BVgroup is more mobile than the other groups ($p<0,001$).Leptin levels in the prefrontal cortex were significantly higher in the 0.1mg/kgBV group compared to control and 0.5mg/kg groups($p<0.001$).There were no significant difference between groups in hipocampus and amigdala leptin levels.
CONCLUSION: The results of the study show that BV has a positive effect on behavioral parameters. 0.1mg/kg BV can have a positive effect by increasing the level of leptin in the prefrontal cortex where anxiety and depression-like behaviors are triggered.
Keywords: Leptin, behavioral parameters, bee venom.

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OC-34

The Effects of Different Types of Calorie Restriction on miRNA Profiles in Brain

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AIM: Beneficial effects of calorie restriction (CR) has been shown in variety of pathophysiological conditions such as cancer, cardiovascular diseases, and neurological diseases. However, the molecular mechanism of CR in pathophysiological conditions is unclear. The aim of the study was to compare the miRNA profile in brain tissue of mice applied to two different types of CR methods.
METHODS: Mice were enrolled in ad libitum (AL), chronic CR (15% of CR application compared to AL group, CCR) or intermittent CR (one week 60 % CR application following three weeks AL feeding in cyclic manner, ICR) groups. Brain samples were collected at weeks 81/82 of mouse age. Afymetrix miRNA microarray was used to analyze microRNA expression. For each sample total of 3,195 miRNA was analyzed (ebayes ANOVA, n=3). Transcriptome Analysis Console and ClueGO were used to investigate the roles of miRNAs in specific signaling pathways. For the statistical analyses, Transcriptome Analysis Console 4.0.1 with default settings were used. Targets of DE miRNAs were transferred into Cytoscape plugin Cluego 2.5.4 and Gene Ontology (GO) analysis were performed with Enrichment/Depletion (Two-sided hypergeometric test) with Bonferroni step down.
RESULTS: Total of 28 miRNAs and two precursors were differentially expressed among the different dietary groups. Level of mmu-miR-713 was significantly higher in CCR group compared to the rest. Compared to AL group, total of 56, 223, and 103 validated targets were determined in CCR, ICR-R and ICR-RF groups, respectively. Common GO terms between dietary groups include terms like nervous system development, neurogenesis and generation of neurons.
CONCLUSION: miRNAs may play important roles in the protective effects of CR in transcriptional regulation, dendritic and axonal pathways. Acknowledgement: Supported by TUBITAK (project # 119S238)
Keywords: miRNAs, calorie restriction, transcriptional regulation, brain, neurological pathways.

OC-35

Effects of Spirulina Platensis on Behavioral Parameters and Sirtuin-1 in High Fat Diet-Induced Obesity in Rats

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AIM: Long term high fat diet causes obesity. Obesity-induced metabolic changes can lead learning and memory impairment and behavioral disorders. Spirulina platensis (SP) is a blue-green microalgae, which has antioxidant, antiinflamatory, neuroprotective, antidiabetic and antiobesity properties. Sirtuins are enzymes, which modulate various biologic functions as gene transcription, cell differentiation, energy metabolism, aging and apoptosis. This study was made to investigate the dose-dependent effects of SP on learning and memory, behavior and brain Sirtuin-1 levels in high fat-induced obesity.
METHODS: Four weeks old Sprague Dawley male rats were used in the study, rats were divided into four groups; control, high fat diet (HFD), HFD+SP150 (150 mg/kg/day), HFD+SP450 (450 mg/kg/day). Rats except for the control group exposed to 60% high fat diet along 12 weeks. Last 6 weeks SP or vehicle administered by oral gavage. Learning and memory was assessed using the Morris water maze (MWM) and behavioral performance was assessed using the T-maze, Open field test (OFT) and forced swimming test (FST). After the behavioral tests, rats were sacrificed and brain regions were taken. Sirtuin-1 levels were measured by Elisa method in prefrontal cortex, hippocampus and amygdala tissues.

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RESULTS: HFD group gained more weight than control and HFD+SP450 groups ($p<0.05$). HFD for 12 weeks did not induce cognitive impairment in MWM test. The open field test showed no significant difference between groups in distance traveled. No significant difference was found between groups in the T-maze test. In FST, immobile time for HFD group significantly higher than control and HFD+SP450 group ($p<0.05$). No significant difference was found between the groups in Sirtuin-1 levels in the brain regions.
CONCLUSION: The results of our study show that chronic high fat diet and SP treatment does not affect learning and memory function. HFD+SP450 treatment positively affects chronic high fat-induced depressive behavior without affecting Sirtuin-1 levels.
Keywords: Spirulina platensis, obesity, learning, behaviour, sirtuin.

OC-36

Is quail egg a potential endocrine disrupter?

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AIM: Since we have observed quail egg consumption (QE) in the history of patients with precocious puberty, we investigated possible endocrine disrupting effect of QE by a retrospective study in girls with premature thelarche (PT) as well as by an experimental study conducted in QE-fed rats.
METHODS: The medical records of 6-8-year-old female patients (n=55) with PT were examined retrospectively, while type-1 diabetic female patients at 6-8 years with good glycemic control (n=54) were taken as control group, and were questioned about amounts of QE intake in their diets. Female Sprague-Dawley rats were orogastrically given QE (300, 1000 mg/kg/day) or 17β-estradiol (E2, 50 μg/kg/day) or tap-water (control) starting by postnatal 14th day and continued until 30th day. Anthropometric measurements and vaginal opening dates were recorded, vaginal smears were analyzed. Following euthanasia, serum FSH and estradiol levels were measured. Uterus and vagina tissues were examined histologically. Data were compared using chi-square (patients) and one-way ANOVA (rats).
RESULTS: QE comsumption in the diabetic control patients was either mild (20.4%) or moderate (74%), while a heavy QE consumption (20%) along with moderate (21.8%) and mild (18.2%) intake was present in PT-patients ($p<0.01$). Uterine and ovarian weights of E2- and QE-treated rats were higher than those of the control group. Vaginal smear analysis and histological analysis confirmed elevated counts of cornified cells and high numbers of endometrial glands, indicating enhanced estrogenic activity in QE-treated ($p<0.05$) and E2-treated ($p<0.001$) rats, while FSH and estradiol levels were increased in both groups ($p<0.05$-0.01).
CONCLUSION: Both our clinical data showing a relation between a high quail egg consumption and a facilitated pubertal development and our experimental findings demonstrating increased uterine and ovarian weights, elevated serum estradiol and FSH levels, cornification of vaginal epithelium suggest that quail egg needs to be further investigated as a potent endocrine disrupter.
*Supported by BAPKO (SAG-C-TUP-130219-0049).
Keywords: Quail eggs, precocious puberty, endocrine disruptors.

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OC-37

The Effect of Melatonin on Trace Element Distribution in Muscle Tissue at Experimental Type 1 Diabetes

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AIM: In this study, the effects of melatonin application on copper (Cu), iron (Fe), manganese (Mn), selenium (Se), and zinc (Zn) trace elements levels were evaluated in muscle tissue of streptozotocin-induced diabetic rats.
METHODS: Forty female Spraque Dawley rats were divided into 4 groups. (Control group-C, Diabetic group-D, Melatonin Applied Group-M, and Melatonin Applied Diabetic group-D+M) of 10 rats in each. C group was fed with standard rat provender, and did not receive any treatment. A single dose of 50 mg/kg streptozotocin was given intraperitoneally (i.p.) the rats in the D and D+M groups. The dose of 10 mg/kg/day melatonin was given to the D+M and M groups i.p for 6 weeks. Trace elements levels in muscle tissue were evaluated by using inductively coupled plasma optical emission spectrophotometer (ICP-OES).
RESULTS: Cu level was significantly increased in the muscle tissue of the rats in the D and D + M groups compared to the C and M groups ($p<0.05$). Zn and Fe levels were significantly increased in the D, M and D + M groups in the muscle tissue ($p <0.05$). There was no significant difference in Se and Mn levels among groups ($p>0.05$).
CONCLUSION: As a result, it was observed that diabetes affected trace element levels in the muscle tissue, and melatonin application increased significantly trace element levels. These results demonstrate that using melatonin as a supplement may decrease tissue damage in diseases with systemic effects such as diabetes.
Keywords: Melatonin, muscle, type 1 diabetes, trace elements.

OC-38

Effect of Intracerebroventricular MOTS-c Infusion on Peripheral Uncoupling Proteins in Rats

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AIM: MOTS-c is a peptide which reduces obesity and insulin resistance and plays an active role in cell metabolism. Uncoupling Proteins (UCPs) synthesized from the inner membrane of the mitochondria are known to play a role in the function and cellular energy regulation of the mitochondrial membrane. We have previously reported that ICV MOTS-c infusion did not cause any significant change in body weight despite increasing food consumption in rats. The previous findings suggest that this peptide may have a role role in the metabolic rate and energy balance. Therefore, the present study was conducted to investigate effects of intracerebroventricular (ICV) infusion of MOTS-c on peripheral UCPs in male rats.
METHODS: In this study, a total of 40 adult male Wistar rats were divided into 4 groups with their body weights close to each other (n=10). The rats outside the control group were infused to the lateral ventricles using osmotic mini pumps in a volume of 5 µl / hour (artificial Cerebro Spinal Fluid, CSF in the Sham group, 10 and 100 µM MOTS-c in the administration groups). After 14 days, the rats were decapitated and muscle, white and brown adipose tissue samples were collected. UCP1 and UCP3 mRNA levels in white and brown adipose tissue and muscle tissue were determined by RT-PCR method. In addition, tissue protein levels of these genes were determined by Western Blot method.
RESULTS: It was determined that UCP1 mRNA and protein levels in white and brown adipose tissue and UCP3 mRNA and protein levels in muscle tissue significantly increased due to MOTS-c infusion ($p<0.05$).
CONCLUSION: The results of the present study suggest that MOTS-c plays an important physiological role in energy metabolism that may be mediated via peripheral

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UCPs. How MOTS-c causes such effects and underlying mechanism(s) remain to be further elucidated.
Acknowledgment: This study was supported by TUBITAK (Project # 116S744) and Inonu University BAP (Project # TYL-2019-2026).
Keywords: MOTS-c, UCP1, UCP3, BAT, WAT.

OC-39

The Effect of Experimental Hyperthyrodism Treatment on Adropin, Asprosin and Preptin Levels in Rats

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AIM: Thyroid hormones have important roles in normal development and energy regulating mechanisms as well as signaling mechanisms that affect energy consumption through central and peripheral pathways. The aim of this study was to determine the effects of experimental hyperthyroidism on adropin, asprosin and preptin levels in rat.
METHODS: The study was performed on the 38 male Wistar-albino rats. Experiment groups were designed as follows. 1-Control, 2 Hyperthyroidism; Rats were made with hyperthyroidism by 3 weeks L-thyroxine (0,3 mg /kg/day). 5-Hyperthyroidism +PTU; Animals were made hyperthyroisim by L-thyroxine as groups 4, then 1 week PTU was applied to treatment of hiperthyrodism. The end of supplementation animals were sacrified and blood samples were collected for FT3, FT4, adropin, asprosin, preptin analysis.
RESULTS: FT3 ve FT4 levels were reduced significantly in hyperthyroidism (P<0.001). Hipothyrodism led to reduction in adropin, asprosin and preptin levels. Hyperthyroidism reduced adropin and preptin levels (P<0.001).
CONCLUSION: The results of study show that experimental hyperthyroidism leads to significant changes in adropin and preptin levels. However, PTU caused further reductions in asprosin and preptin levels after hyperthyroidism.

Keywords: Hyperthyroidism, Preptin, Adropin, Asprosin.

OC-40

Preliminary Study: Investigation of the Effect of Swimming Exercise on Browning of Perirenal Fat Tissue in Rats with Metabolic Syndrome

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AIM: The alteration of white adipose tissue (WAT) ''browning'', a change of white into beige fat, has been considered as a new therapeutic strategy to treat obesity. In this study, we investigated the browning effects of 6-weeks swimming exercise in rats with metabolic syndrome (MetS).
METHODS: Male Wistar rats (2-3 months old) were randomized into MetS, control (C), MetS+exercise (M-E), Control+exercise (C-E) group. We evaluated the browning effects of 6-weeks swimming exercise in perirenal adipose tissue. Rats with M and M-E groups were fed a diet containing 20% fructose for 16 weeks. All animals were fasted for 48 h after the swimming protocol and were sacrificed. Perirenal adipose tissues of the rats were taken 2 days after the end of the exercise. Histological examination was performed with hemotoxylin eosin staining. UCP-1, CD137 gene expressions were examined by real-time PCR.
RESULTS: Histological examination showed no difference between C and MetS groups regarding brown adipose tissue (BAT), whereas in the C-E and M-E groups 5-fold increase in BAT was detected. The expression of UCP-1 and CD137 were increased in M-E rats compared to MetS group (1.50 and 3.22 fold change respectively).
CONCLUSION: Our results showed that 6-weeks of swimming exercise seems to induce remodeling of WAT to BAT in rats with MetS by increasing gene expressions that regulate thermogenic modulations in perirenal adipose tissue.

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Acknowledgement: This study was supported by PAU-BAP (Project 2019SABE005).
Keywords: adipose tissue, browning, swimming exercise, UCP-1.

OC-41

Does Adipose Tissue as an Endocrine Organ Play A Role in Pathologies of The Spinal Dura Mater?

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AIM: Adipose tissue is a dynamic organ that is the primary site of storage for energy but also have diverse functions such as modulation of tissue growth and tissue repair or regeneration process. Numerous surgical techniques and synthetic or autologous adjuvant materials have been used to treat spinal duramater pathologies and complications. Some of these techniques and materials are unsatisfactory and cause further complications. Use of an adipose graft was previously recommended by authors, who reported the effectiveness of it for the protection and repair of the spinal dura. Here we described safe and effective method by using autologous free adipose graft harvested from the subcutaneous tissue to treatment of Tarlov cyst that is cerebrospinal fluid-filled nerve root cyst.
METHODS: A 45 years old male patient presenting with radicular pain related to his right leg. Neurological examination was revealed motor and sensory deficit on the right side. Radiological examination revealed a huge sacral Tarlov cyst. The patient underwent one level hemilaminectomy. The anterior wall of cyst was removed and then crushed nerve roots was meticulously dissected from the cyst wall step by step under operation microscope. Free fat grafts that harvested from the lateral thigh were placed to the cyst cavity and around the nerve roots. Fibrin glue was then applied between the fat grafts to keep them in place and hold them together.
RESULTS: Neurological functions in patient significantly improved in comparison to the pre-operative investigation. Disturbances in sensation, abnormal reflexes was no longer in evidence and no evidence of postoperative a cerebrospinal fluid fistula within the follow-up period.
CONCLUSION: The use of autologous free adipose tissue grafts is a safe and effective technique to the treatment of Tarlov cysts.
Keywords: Adipose tissue, tissue healing, tarlov cyst.

OC-42

Is Duration of Menstruation Correlated with the Activity of Stress Axes?

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AIM: The sympathetic branch of the autonomic nervous system and the hypothalamic pituitary adrenal axis (HPA) are activated in response to stress. In order to measure the activity of the autonomic nervous system, heart rate variability (HRV) is used. For determination of HPA activity, salivary cortisol is used for short-term, but hair cortisol is used for long-term. Purpose of the current study was to find out effect of duration of menstruation on the stress axes.
METHODS: For this purpose, 40 women between the ages of 18-24 participated in the study following ethical approval from the Malatya Clinical Research Ethics Committee. Following a questionnaire, two groups were formed as duration of menstruation being ≤ 6 or > 7 days. Hair samples were taken from the posterior vertex region. Salivary samples were collected at 30 minutes after awakening, at noon and at midnight before going to sleep. For determination of heart rate variability, a 5-minute ECG was taken and heart rate variability was calculated. Kruskal-Wallis test was used for statistical analysis. Data were presented as median (min-max), and $p<0.05$ was considered significant.
RESULTS: The cortisol level in hair and saliva did not differ in participants with a menstruation period of ≤ 6 and >7 days. Heart rate variability parameters; the rates of SDNN, CV%, TP, VLF, LF were lower for women having a menstruation duration of >7 days.
CONCLUSION: No relationship was found between the length of menstruation and acute and chronic levels of cortisol, suggesting that HPA axis activity was not

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changed. On the other hand, as lower HRV (SDNN, %CV, TP, VLF, LF) has been associated with bad health outcome, lower HRV in women having longer menstruation is a point needing to be emphasized.

Keywords: Hair cortisol, Salivary Cortisol, Menstrual duration, HPA, HRV.

OC-43

Effects of Irisin on Ghrelin Levels in High-Fat Diet-Induced Obese Female Rats

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AIM: Ghrelin is an orexigenic peptide and primarily produced by the stomach and also hypothalamus and stimulates food intake. It is known that the levels of ghrelin decreases in response to overfeeding in obese people. The varying effects of exercise on the ghrelin levels are known. However, the effects of irisin, an exercise hormone, on the ghrelin levels are unknown in obesity. The purpose of this study was to investigate effects of chronic irisin treatment on serum ghrelin levels in high-fat diet-induced obese female rats.
METHODS: Forty female Sprague Dawley rats (2-3 months and 200-250 g) were randomly divided into four groups as control, sham-operated, obese and obese+irisin. To obtain high-fat diet-induced obesity models all animals in obese and obese+irisin groups were fed with a high-fat diet, while control and sham-operated rats were fed a control diet during the experiment (about 16 weeks). After 12 weeks of diet exposure, Lee index was measured for experimental obesity validation. Then irisin was subcutaneously administered with osmotic minipumps at the dose of 100 ng/kg/day for 28 days to obese+irisin group. The serum levels of ghrelin were determined with ELISA method at the end of the experiment.
RESULTS: When compared to the control or sham-operated groups, it was observed that serum ghrelin levels significantly decreased in both the obese and obese+irisin group ($p<0.05$). Furthermore, there was a significant decline in the serum ghrelin levels in obese+irisin group compared to obese group ($p<0.05$).
CONCLUSION: The present findings suggest that exogenous irisin administration can be a therapeutic agent for obesity by reducing the levels of ghrelin.

Keywords: Irisin, ghrelin, obesity, female rat.

OC-44

Effect of Apelin on Anxiety Like Behaviour in Male Rats

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AIM: Apelin is a novel peptide in central nervous system and has many physiological impacts. In this study, it is aimed to investigate the possible effect of apelin treatment on anxiety like behaviour in socially isolated rats.
METHODS: Anxiety was constituted by social isolation model in all animals. Young male rats (age of 4 weeks) separated from their mothers for 8 weeks. Anxiety status was evaluated by elevated plus maze test and open field test at the end of 6th week and pups determined anxiety were involved the letter experiments. Rats were randomly divided as control, apelin, anxiety and anxiety+apelin group. After administration of subcutaneous apelin via osmotic minipumps for 14 days, behavioural tests were applied to the all animals again. One-way ANOVA was used for statistical evaluation.
RESULTS: Apelin administration increased grooming number, mobility time and total distance travelled compared to anxiety group in open field test ($p<0.05$). In the elevated plus maze test, number of entrance to the open arms and time spent in open arms were significantly decreased in anxiety group and apelin administration increased this parameter compared to anxiety group ($p<0.05$). Additionally, time spent in closed arms were decreased in apelin group but there was no statistically significance.
CONCLUSION: Social isolation stress apparently induces anxiety-like behaviours in male rats. We can also conclude that apelin treatment may bring about

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anxiolytic affect. The exact mechanism of this effect needs to clarify.

Keywords: Apelin, Anxiety, Rat, Social isolation, Elevated plus maze test.

Poster Communications

PC-01

L-Lactate in Rat Astrocytes Originates from Glycogen Stores

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AIM: Astrocytes are numerous neuroglial cells of the central nervous system with ideal anatomical position between neurons and vasculature, which enables them to provide glucose for neurons. Neuronal networks require additional 20 % of their normal energy consumption during cognitive efforts, mediated by chemical messengers including noradrenaline (NA). NA targets astroglial aerobic glycolysis, the process of which the end-product is L-lactate. Biochemical studies revealed that astrocytes exhibit a prominent glycogen shunt, where a part of glucose molecules entering the cytoplasm are transiently incorporated into glycogen.
METHODS: Here, we studied single astrocyte L-lactate metabolism using the FRET nanosensor Laconic and tested how is the noradrenaline-induced cytosolic L-lactate ([lactate]i) increase influenced by: i) inhibiting glycolysis by 2-deoxy-D-glucose (2-DG, 3 mM), a molecule that enters cytosol but inhibits the glycolytic pathway; by ii) inhibiting glycogen degradation by 1,4-dideoxy-1,4-imino-d-arabinitol (DAB, 300 μM), a potent inhibitor of glycogen phosphorylase; and by iii) the application of 3-nitro-propionic-acid (3-NPA, 1 mM), and inhibitor of the Krebs cycle.
RESULTS: The results of these studies revealed that D-glucose uptake is essential for the NA-induced increase in [lactate]i, and that it exclusively arises from the glycogen degradation, indicating that most, if not all, D-glucose molecules entering the cytosol transit the glycogen shunt. Moreover, at these experimental conditions of defined transmembrane D-glucose gradient, the glycolytic intermediates are not only used to produce L-lactate, but also significantly support oxidative phosphorylation as

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revealed by the elevation in ([lactate]i) while the Krebs cycle was inhibited.
CONCLUSION: We conclude that glycogen degradation and formation are targets of noradrenergic stimulation. We showed that all lactate after NA stimulation derives from glucose originating from glycogen shunt. We found that oxidative metabolism is equally important since Krebs cycle blockage results in increased lactate formation.
Keywords: Lactate, noradrenaline, glycogen, astrocytes, aerobic glycolysis, FRET.

PC-02

Angiotensin II Type 1 Receptor Blockade Suppresses NLRP3 Inflammasome and IL-1β, While Increasing BDNF Level in Ovariectomized Female Rats

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AIM: Bilateral ovariectomy increases the risk of neurodegenerative and affective disorders prior to the age of natural menopause in women. Many studies have reported a relationship between estrogen depletion and activation of the renin-angiotensin system. The nucleotide binding and oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome is implicated to linkage in development of neuroinflammation. Activation of the NLRP3 inflammasome subsequently activates caspase-1 and induces the secretion of IL-1β. The brain-derived neurotrophic factor (BDNF) involved in synaptic plasticity and multiple forms of memory formation. Estradiol regulates BDNF expression in a number of brain areas including hippocampus. Several studies have demonstrated that angiotensin type I receptor blockers (AR1Bs) exerts neuroprotective actions. In this study, we investigated the effect of AT1RB on NLRP3, IL-1β, BDNF and CREB levels in the hippocampus and prefrontal cortex of ovariectomized (OVX) female rats.
METHODS: Thirty two Wistar albino female rats were randomly divided into 4 groups (n = 8); Control, ATIRB treated control, OVX and OVX+AT1RB treated group. AT1RB was administered at a dose of 40mg/kg/ day by intragastric gavage. After 14 days, NLRP3, IL-1β, BDNF and CREB were analyzed in hippocampus and prefrontal cortex of rats by ELISA. Statistical analyses of data were performed by Kruskal Wallis and Dunn Tests.
RESULTS: Our results showed that OVX resulted in an increase of NLRP3 inflammasome and IL-1β in the hippocampus. AT1RB supressed the levels of NLRP3 and IL-1β in OVX rats. The levels of CREB did not change both by OVX and AT1RB treatment. OVX resulted a decreased in BDNF levels in the hippocampus. AT1RB produced a significant increase in hippocampal BDNF levels in OVX rats.
CONCLUSION: Our data indicate that AR1Bs have neuroprotective potential in the loss of ovarian function by inhibiting the NLRP3 inflammasome and increasing BDNF levels.
Keywords: Angiotensin II, NLRP inflammasome, BDNF, ovariectomy.

PC-03

The Effects of Melatonin Treatment on Postoperative Induced Cognitive Dysfunction in Aged Rats: Involvement of Oxidative Stress, PSD95 and CaMKII

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AIM: Geriatric patients who are exposed to major surgery are known to be sensitive to neurocognitive disorders, e.g., postoperative cognitive dysfunction (POCD) and delirium. Substantial evidence showed that the pathogenesis of POCD is associated with neuroinflammation, oxidative stress and pro-inflammatory cytokines. Melatonin is known to be free radical scavenger, anti inflammatory and antioxidant actions. The goal of the this study was to investigate the effects of melatonin on cognitive function, oxidative stress markers and synaptic proteins in hippocampus of POCD rats.
METHODS: Aged male Wistar rats (n=40, 18-24 months) were randomly divided into four groups. Control, control+melatonin treatment, surgery, surgery+melatonin treatment. To mimic abdominal surgery the intestine was exteriorized and strongly rubbed for 30 sec. Melatonin treatment was applied 10 mg/kg (ip). One week after surgery cognitive functions were assessed using the novel obje recognition test (NOR). The levels of malondialdehyde (MDA), postsynaptic density protein 95 (PSD95) and Calcium/ calmodulin dependent protein

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kinase II (CaMKII) were measured in the synaptosomes of hippocampus. Statistical analyses were performed by using Krusskal-Wallis test and post-hoc Dunn test.
RESULTS: Our results showed that surgery impaired cognitive function in NOR test. Surgery rats spent less time exploring the novel object than the familiar object compared to the control group. Surgery inceased MDA levels and decreased PSD95 and CaMKII levels in hippocampus. Melatonin treatment alleviated surgery-induced impairment of novel object discrimination and reduced MDA levels. Melatonin treatment increased synaptic protein levels in hippocampus.
CONCLUSION: Our findings suggest that melatonin treatment may be potential agent which may protect against surgery induced cognitive dysfunction in aged patients.
Keywords: Melatonin, post-operative cognitive dysfunction, aged rats, oxidative stress, PSD95.

PC-04

Effects of Estrogen and Progesterone on Biomarkers of Neurogenic Inflammation Underlying Migraine in Rats

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AIM: AIM: Neurogenic inflammation is known to be responsible for migraine pathophysiology. Calcitonin gene-related peptide (CGRP) and substance P (SP) released from trigeminovascular system are crucial triggers of neurogenic inflammation. Migraine is approximately three times more frequent in women than in men. Although it seems that estrogen withdrawal during menstrual cycle may be responsible for this gender difference, its mechanisms remain unclear. We investigated effects of female-sex hormones estrogen and progesterone on plasma levels of CGRP and SP in rats of both sexes.
METHODS: Adult Wistar rats were divided into 3 general groups as intact male, female and ovariectomized female. Bilateral ovariectomy was performed. General groups were separated into 4 subgroups (n=7) as control, estrogen, progesterone and combination (estrogen+progesterone). Rats received intraperitoneally daily 0.2 ml vehicle, 1mg/kg 17β-estradiol, 8 mg/kg progesterone and 17β-estradiol+progesterone for 5 days, respectively. After 5 days, venous-blood was collected. Plasma CGRP and SP levels were measured using ELISA. Data were compared by using One-way ANOVA.
RESULTS: Compared to their control groups, 17β-estradiol decreased CGRP levels in male-estrogen group ($P<0.05$) and SP levels in ovariectomized-estrogen group ($P<0.05$), did not significantly change CGRP and SP levels in female-estrogen group. While progesterone increased CGRP and SP levels in female-progesterone group ($P<0.001$), did not significantly alter them in both male and ovariectomized-progesterone group. While the combination decreased CGRP and SP levels in male-combination group ($P<0.001$, $P<0.05$), increased them in female-combination group ($P<0.05$, $P<0.001$). The combination did not significantly change CGRP and SP levels in ovariectomized-combination group rats.
CONCLUSION: Estrogen reduced levels of neurogenic inflammation markers including CGRP and SP in both sexes. Progesterone induced an increment in CGRP and SP levels in females. Our results suggest that estrogen may alleviate neurogenic inflammation underlying migraine via modulation of CGRP and SP release, and progesterone may exacerbate it by inducing CGRP and SP release.
Grant-number: 2017.08.02.1258 (BAIBU-BAP).
Keywords: Estrogen, progesterone, migraine, CGRP, SP, neurogenic inflammation.

PC-05

Unraveling the Role of Astroglia in the Mechanism of Antidepressant Action of Ketamine

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AIM: Ketamine (KM), an anaesthetic and psychotomimetic drug, exerts rapid, potent and long-lasting antidepressant effect, albeit the cellular and molecular mechanisms of this action are incompletely understood.

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Besides targeting neuronal NMDARs, KM also modulates the function of astroglia. We thus elucidated the effect of (sub)anaesthetic doses of KM on stimulus-evoked calcium (Ca^{2+}) signaling, fusion pore activity of secretory vesicles and mobility of vesicles carrying the inward rectifying potassium channel (Kir4.1) in astroglia.
METHODS: The effect of KM on ATP-evoked Ca^{2+} signaling was examined by measuring the Fluo-4 fluorescence in KM-treated and non-treated astrocytes. High-resolution patch-clamp membrane capacitance measurements were used to determine the fusion pore activity of secretory vesicles. The spontaneous mobility and plasmalemmal localization of Kir4.1-EGFP in pKir4.1-EGFP-transfected astrocytes labeled by styril dye FM4-64 was determined by confocal microscopy.
RESULTS: The ATP-evoked peak Ca^{2+} responses were diminished in KM-treated astrocytes with ATP mobilizing ~3.3-fold less Ca^{2+} than in non-treated controls (P <0.001). Ketamine-evoked increase in vesicle bursting activity correlated well with a decrease in irreversible vesicle fission from the plasmalemma (R = 0.93 for increasing KM incubation time and R = 0.99 for increasing [KM]). Already short, 30 min KM treatment reduced directional mobility of Kir4.1-positive vesicles. The apparent surface localization of Kir4.1 at astroglial plasmalemma decreased from 56% in non-treated controls to 43% (P <0.05) and 33% (P <0.05) in astrocytes treated with 2.5 and 25 μM KM, respectively.
CONCLUSION: Diverse but not mutually exclusive mechanisms of ketamine action may synergistically evoke changes in synaptic functional plasticity, leading to sustained strengthening of excitatory synapses, necessary for antidepressant effects.
Acknowledgements: The authors acknowledge financial support from the Slovenian Research Agency (P3-310, J3-6790, J3-6789, J3-7605 and J3-9266). We kindly thank Professors Serguei Skatchkov and Misty Eaton (Central University of the Caribbean) for sharing the plasmid Kir4.1-EGFP.
Keywords: astroglia, ketamine, calcium signalling, endocytosis, exocytosis, Kir4.1

PC-06

Reversible Exocytosis of Larger Vesicles and Inhibition of Endocytosis Underlie Interferon γ Induced Translocation of MHC Class II Molecules to Astrocyte Surface

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AIM: Interferon γ (IFNγ) is an inflammatory cytokine that induces expression and plasmalemmal localization of major histocompatibility complex class II molecules (MHCII) in several cell types including astrocytes. MHCII is essential for antigen presentation to T cells and thus immune responses. We here examined vesicular mechanisms involved in delivery of MHCII to plasmalemma and their retention at the surface of IFNγ-treated astrocytes.
METHODS: We treated cultured neonatal rat astrocytes with 600 U/ml of IFNγ for 48h. Confocal microscopy was used to evaluate the quantity and plasmalemmal localization of immunolabeled MHCII and high-resolution cell-attached patch-clamp capacitance measurements were performed to investigate elementary events of exo-/endocytosis.
RESULTS: IFNγ treatment increased the expression of MHCII in astrocytes; the relative proportion of MHCII-immunopositive cell area increased by ~eightfold (from 1.5% to 11.6%; P<0.001). The plasmalemmal localization of MHCII was detected only in live, non-permeabilized astrocytes treated with IFNγ. In these astrocytes larger vesicles underwent reversible exocytosis and the frequency of full endocytosis was reduced. Stimulation with 100 μM ATP increased the free intracellular Ca^{2+} concentration and modulated exo-/endocytotic activity; the frequencies of reversible and full exocytosis increased, whereas the frequency of full endocytosis decreased. ATP-evoked alterations of exo-/endocytotic activity were largely conserved in IFNγ-treated astrocytes.
CONCLUSION: IFNγ induces expression and plasmalemmal localization of MHCII in astrocytes. Larger vesicles preferentially enter reversible exocytosis and deliver MHCII to the plasmalemma of IFNγ-treated

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astrocytes. Concomitant inhibition of full endocytosis seemingly prolongs MHCII exposure at the cell surface of IFNγ-treated astrocytes. The modulation of elementary exo-/endocytosis by ATP is largely intact in IFNγ-treated astrocytes.

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Keywords: Astrocyte, interferon γ, MHCII, patch-clamp, membrane capacitance.

PC-07

Mast Cell Activation and Proinflammatory Cytokines Contribute to Persisting Inflammation in Children with Cerebral Palsy

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AIM: Cerebral palsy (CP) is a common disabling disease characterized by non-progressive brain injury. It is well established that inflammatory processes during intrauterine life or before 3 years of age are related to pathophysiology of CP but, inflammatory processes on the persisting of disease in children above 3 years of age are unknown. We explored the association between mast cells, proinflammatory cytokines and the persisting of disease in children with CP above 3 years of age.

METHODS: Venous blood samples were collected from 30 CP patients and 26 healthy volunteers aged 3-18 years. Serum levels of proinflammatory cytokines including IL-1β, IL-6 and IL-9, and biomarkers of number and activation of mast cells including tryptase beta-2 and histamine were determined using ELISA. Data were compared by Mann-Whitney U test.

RESULTS: IL-1β, IL-6 and histamine levels were higher in patients compared to controls ($p<0.05$), but significant difference between groups was not determined for IL-9 and tryptase beta-2 levels ($p>0.05$). IL-1β and IL-6 levels were increased in female patients compared to female and male volunteers in control ($p<0.05$). IL-9 levels were elevated in female patients compared to female volunteers in control ($p<0.05$). IL-6 levels were elevated in preadolescence patients compared to controls ($p<0.05$). IL-1β, IL-6 and IL-9 levels were elevated in preadolescence female patients compared to both preadolescence and adolescence female volunteers in control ($p<0.05$).

CONCLUSION: Increased levels of proinflammatory cytokines (IL-1β, IL-6 and IL-9) and mast cell activation biomarker (histamine) indicate that inflammation contributes to the persisting of disease in children with CP above 3 years of age. Enhanced systemic inflammatory response is more potent in female patients than male patients, however it declines during adolescent age. Therefore, the increase of estrogen hormones in line with puberty in female patients may be responsible for the decline in inflammatory response due to anti-inflammatory characteristics of estrogens.

Grant-number: 2019.08.02.1415 (BAIBU-BAP).

Keywords: Cerebral palsy, inflammation, mast cells, cytokines, gender.

PC-08

The Influence of Maternal Separation on Depressive Symptoms and Energy Homeostasis in Young Adult Male Rat Offspring Subjected to Chronic Social Defeat Stress

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AIM: Early life stress is known to increase the risk to develop stress. Moreover, chronic social defeat stress (CSDS) has been often used to induce depressive like behavior in rodents. Accordingly, the present study was undertaken to test whether maternal separation would amplify comorbidity of depression and energy homeostasis disturbance in response to chronic social defeat stress in adulthood.

METHODS: Male Wistar rat offsprings were randomly divided into maternal separation (MS) and non-stress (non-STR) groups. The MS animals were separated from their mothers during postnatal days 1 to 14. At puberty

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(day 50), the animals of each group were underwent CSDS for three weeks. Thus, totally, there were 4 groups, nonSTR, MS, CSDS and MS-CSDS (n=10/group). The offspring body weight, length and food intake were determined weekly, and the Lee index was calculated. At the end of the CSDS period, forced swimming test (FST) and sucrose preference test (SPT) were done to evaluate depressive like behaviors. Finally, the animals were decapitated and their blood was collected to measure plasma concentrations of leptin. Also intra-abdominal fat was removed and weighed out.
RESULTS: Lee index, food intake and intra-abdominal fat mass were decreased in both CSDS and MS-CSDS groups compared to non-STR animals. Moreover, the preference of 1% sucrose solution and active coping strategy in the FST were decreased in CSDS group, while in the MS-CSDS group, no significant change was noted. Plasma leptin levels did not change in response to stress.
CONCLUSION: The results of the present study show that early life exposure to mild stressful experiences can trigger adaptive processes that possibly promote resistance to the subsequent depressive like behaviors induced by social challenges later in life.
Keywords: Maternal separation, CSDS, Depression, Energy homeostasis.

PC-09

Manipulation of Kiss1 Neurons by Optogenetic Method in Experimental Alzheimer's Disease Model

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AIM: To investigate effects of acute activation and inhibition of kisspeptin neurons on spatial learning and memory by using optogenetic methods in Alzheimer's Disease (AD) model of female kiss-cre transgenic mice.
METHODS: AD model was induced by infusion of amyloid beta (Aβ) in dentat gyrus region of female Kiss1-CreGFP transgenic mice. CRE-dependent virus (AAV-Flex-ChR2-YFP /- iCholoC2A-dsRED) was injected into the hypothalamic Arcuate Nucleus (ARC) by stereotaxic method for optogenetic manipulation of kisspeptin neurons. Concomitant with the virus injection, a fiber optic cable with a diameter of 200 μm was unilaterally placed 0.5 mm above ARC. Three weeks after implantation, effects of activation and inhibition of kisspeptin neurons in ARC region on cognitive functions were examined by Morris's water maze (MWM). Activation and control groups were given 10 Hz light stimulation, 1 second every 3 seconds for 5 minutes. In the inhibition group, continuous 2 mW light stimulation was applied for 5 minutes. One-Way ANOVA was used for statistical analysis of the data.
RESULTS: Confocal microscopy analysis revealed that the virus injections were in the correct coordinates, and optogenetic stimulations were confirmed with c-FOS staining. Cognitive functions as evaluated by MWM did not show significant changes among the groups.
CONCLUSION: These preliminary findings suggest that acute activation and inhibition of kisspeptin neurons in experimental AD model did not have any significant effect on spatial learning and memory. We plan to further test the hypothesis with larger group size and using a different cognitive test since it is thought that ferrule implantation could be a limiting factor on swimming ability of the animals.
Acknowledgement: This study was supported by TÜBİTAK (Project # 115S327).
Keywords: Kisspeptin, Alzheimer's Disease, Optogenetics, Morris Water Maze.

PC-10

Effect of the Inhibition of Rock Signaling on the Hippocampal Metaplasticity

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AIM: The present study investigates the effect of Rho-Kinase (ROCK) inhibitor Fasudil on the formation of metaplasticity (MP) in the hippocampus without pathological disorders.
METHODS: Control (C, n=8) and Fasudil (F, n=8) groups were composed of Wistar-Albino male rats. Our

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study was approved by the ethics committee of Erciyes University, numbered 18/090. The anesthetized animal skull was fixed to the stereotaxic system and stimulated with electrode inserting to the perforating path. Serum physiological or Fasudil were infused into the dentate gyrus. Metaplasticity was induced by delivering high frequency stimulation (HFS) 5-min after low-frequency stimulation. Population Spike (PS) amplitude and Excitatory Post Synaptic Potential (EPSP) slopes were compared between groups with Student-t test.
RESULTS: Fasudil infusion eliminated EPSP slope inhibition both in post-tetanic (F=%126,86±5,27, C=%115,29±8,87, $p<0.05$) and maintenance periods (F=%117,40±4,58, C= %80,81±6,76; $p<0.05$). This change reached statistically significant levels. There was no significant difference in PS amplitudes between groups at post-tetanic period (F=%180.93±18.34, C=%201.19±14.09 $p>0.05$), while fasudil infusion resulted in the increased PS amplitude at the maintenance period (F=%173.13±16.30 C=%162.78±12.79, $p>0.05$). However, this change did not reach statistically significant levels.
CONCLUSION: Present results may suggest the contribution of ROCK-signaling pathway to metaplasticity of synapses.
Keywords: Fasudil, hippocampus, metaplasticity, Rho-kinase.

PC-11

Regulation of mTOR pathway, Akt and Erk1/2 activity by Melatonin and Rapamycin in Glioblastoma *in vitro*

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AIM: Glioblastoma multiforme (GBM) is the most prevalent brain tumor with high mortality rates and poor prognosis. Various intracellular pathways including the mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K)/Akt/mechanistic target of rapamycin (mTOR) pathways are frequently deregulated in GBM. Melatonin (secreted by the pineal gland) has been shown to have anti-cancer effects. In our study, effects of melatonin and mTOR inhibitor rapamycin on the regulation of MAPK and Akt/mTOR pathways were investigated in vitro.
METHODS: The human GBM cell lines A172 and U87 were maintained in DMEM containing 10% FBS and 1% PSN at 37°C under humidified atmosphere containing 5% CO2. The cells were treated with rapamycin and melatonin either alone or in combination for 24 and 48h in cell culture dishes. Proteins were extracted and subsequently activities of Akt/mTOR and MAPK pathways determined by western blotting. Two-way ANOVA was utilized for statistical analysis.
RESULTS: Rapamycin significantly reduced the mTOR pathway activity ($p<0.05$) in both cell lines without altering the Akt and Erk1/2 activities at 24h. p-mTOR levels were significantly higher in A172 cells than U87 cells when treated with melatonin ($p<0.05$). p-Erk1/2 levels were also higher in A172 cells than U87 cells, which was independent of melatonin ($p<0.05$). At 48h, p-mTOR levels was significantly reduced by rapamycin compared to control ($p<0.01$). Melatonin-alone significantly reduced p-S6 levels in U87 cells ($p<0.05$). Akt and Erk1/2 activities were not altered, while serum deprivation significantly increased Erk1/2 activity in U87 cells compared to control group ($p<0.001$) at 48h. Only p-S6 levels were significantly lower in A172 cells than U87 cells upon serum starvation ($p<0.001$). p-Erk1/2 levels were significantly higher in serum-starved U87 cells than in serum-starved A172 cells ($p<0.001$).
CONCLUSION: Our results suggest that Akt/mTOR and MAPK pathways may be differentially regulated by melatonin and serum deprivation in GBM cells.
Keywords: Akt, Glioblastoma multiforme, Melatonin, mTOR, MAPK, Rapamycin.

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PC-12

Targeting the Risk of Depression Through Unhealthy Lifestyle: The Relationship Between Insulin Resistance, Physical and Social Activities, Sleep Quality, and Depressive Symptoms in the Prediabetic Period

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AIM: Diabetes evolves out of a prediabetic period, where hyperglycemia is mild, but even so, it has the potential to exert its malignant effects on various structures and functions.The study aims to investigate the potential link between prediabetes and the development of depression according to individual's lifestyle.We evaluated serum lipid profiles, leptin hormone levels, insulin resistance, body mass index, waist/ hip ratio, physical and social activity levels and sleep quality to see their effect on both diseases.
METHODS: 82 patients (M=38/F=44) who were not on medication for regulating blood glucose levels or for depression were recruited by the internal medicine department.Fasting blood glucose levels between 100–125 mg/dL, and/or HbA1c between 5.6-6.9% were considered prediabetic (47.6%) and control group was chosen from normoglycemic individuals (52.4%).Blood samples were collected and anthropometric measurements were done.To access depressive symptomatology “Beck Depression Inventory” was employed and participants reported experiencing mild and moderate symptoms regarded as depressive.All subjects had answered our question sheet related to physical activity levels, participation in social events and sleep quality.
RESULTS: Hyperglycemia, insulin resistance, dyslipidemia, BMI, poor social activity, being physically inactive are factors increasing depression development, but were not sufficient to be considered as statistically significant. Individuals who had high BMI, waist/hip ratio and leptin levels seem to be more aware of the benefits of physical activity for their life. The prevalence of depression was three times higher with individuals who had poor sleep quality (p=0.031, OR 95%, 1.09-9.48).
CONCLUSION: Depression was present in 58.1% of prediabetic patients, whereas 42.9% in normoglycemic patients. When the relationships between leptin/depression and dyslipidemia/depression were assessed, data did not conform to normal distribution (p=0.115). According to the results of the Beck Depression Inventory, there is a tendency towards depression in prediabetic period, but the changes were not statistically significant. Poor sleep quality was found to be a significant factor to increase the progression of depression.
Keywords: Prediabetes, Insulin resistance, Leptin, Depression, Physical and Social Activity, Sleep Quality.

PC-13

Reversible Brain Atrophy After ACTH Treatment In Children With Continuous Spike And Wave During Slow Sleep (CSWS): Case Presentation

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AIM: It is a well-known phenomenon reversible brain atrophy after ACTH treatment in children with epileptic encephalopathy. The aim of this case presentation is to provide clear evidence for formation of reversible brain atrophy related to ACTH treatment and review of the mechanism of ACTH treatment-related reversible brain atrophy in children.
METHODS: A six year- old boy has suffered from frequent generalized tonic-clonic seizures during sleep. His neurologic examination, routine blood, and urine tests and cranial MRI findings were normal. Despite receiving polytherapy his seizures continued, at the same time behavioral disturbances and loss in academic skills have been observed. His sleep EEG examination showed CSWS which is a very special pattern causing a cognitive decline in children. It is known that CSWS is a very important and antiepileptic drug-resistant electrophysiological pattern. If it can not be suppressed or eliminated, it causes cognitive decline and mostly repeated seizures during its course and it can be suppressed with ACTH treatment. For this reason we applied the ACTH to this

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patient one year later his seizures began. The ACTH treatment had to be applied two times for 4 and 2 months respectively with a one-year interval.
RESULTS: At the end of the second ACTH cure, prominent cortical atrophy was seen in his cranial MRI. One year later last ACTH cure, the cranial MRI showed recovering in cortical atrophy especially in the anterior part of the brain.
CONCLUSION: Cortical atrophy related to high serum cortisol level is a well known phenomenon in Cushing syndrome. Hypercortysolemia has effects on the hypothalamic-pituitary-adrenal (HPA)-axis with the structural brain abnormalities and overall brain atrophy even in children. This case is a very well example of ACTH related reversible brain atrophy. Here, we discuss the brain atrophy mechanism related to ACTH treatment in children with CSWS syndrome.
Keywords: ACTH, CSWS, Brain Atrophy, Children.

PC-14

In Silico Analysis on Possible Involvement of Neuropeptides in Peripheral Sensory Neuropathy

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AIM: Peripheral sensory neuropathy (PSN) is a common health problem which may result from traumatic injuries, infections, metabolic problems, inherited causes, toxins and metabolic stress including diabetes. Pathogenesis of PSN is not fully understood yet there is no satisfactory treatment. Clinical experiments are on-going and also recent evidences indicates hope from *in silico* analysis. The aim of this study was to examine possible involvement of neuropeptides in PSN by using bioinformatics tools, by examining the expression levels of genes, known to have neuroendocrine functions through genome analyzes performed in dorsal root ganglia tissues obtained from CL57BL/6J mice, which are known to be susceptible to diabetes (as most common cause of neuropathy), modeled PSN by spared nerve injury.
METHODS: Selected datasets GSE89224[1] and GSE102721[1], associated with hypothesis testing, from GEO (Gene Expression Omnibus) database were re-analyzed separately in R biostatistics program. Expression levels of selected genes, commonly implicated to play a role in neuroendocrine modulations were analyzed *in silico* for each dataset. Based on Benjamini-Hochberg correction, adjusted p-values <0.01 were accepted as significant.
RESULTS: Gene expression levels on each datasets indicated that neuropeptide Y (NPY), galanin (GAL), lectin (LGALS1 and LGALS3), brain-derived neurotrophic factor (BDNF), calcium-calmodulin protein dependent kinase I (CAMK1), inhibin-β (INHBB), insulin-like growth factor (IGFBP3) genes were up-regulated ($p<0.01$) and serotonin receptor 3B (5HTR3B) and estrogen-related receptor (ESRRB) genes were down-regulated ($p<0.01$) in the spared nerve injury groups, compared with sham groups.
CONCLUSION: Results from this *in silico* analysis indicate imbalances in the expression levels (up- and down-regulation) of genes encoding neuropeptides known to be involved in many neuroendocrine process, implicating involvement of impaired neuroendocrine signalling in the pathogenesis of PSN.
Keywords: Peripheral sensory neuropathy, gene expression, neuroendocrine functions, bioinformatics.

PC-15

Effects of Half- or Whole-Night Shifts on Hypothalamo-Pituitary-Adrenal Axis and Autonomic Nervous Systems in Women

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AIM: Scheduled shift workers, especially those working in night shifts, are prone to health problems such as sleep failure, stress and changes in circadian rhythm functions. The aim of this study was to examine the effects of disturbed sleep patterns on hypothalamo-pituitary-adrenal axis (HPA, i.e. corticosterone and cortisol) and autonomic nervous systems (ANS, i.e. alpha-amylase) in females working in dfferent types of shifts.

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METHODS: The participants consisted of female nurses (n=52) working in the University Hospital for at last 5 years (40 night shift workers, 12 day-time workers) or female postgraduate students working in the Faculty of Medicine (all were day-time workers, n=8). The study consisted of 3 groups of female participants: day-time workers (termed "day-time shift," n = 20, between 08:00-16:00 hours), nurses working whole-night shifts (n= 20, between 16:00-08:00 hours) and nurses working half-night shifts (n = 20, between 16:00-24:00 hours). Saliva samples, taken at the beginning, in the middle and at the end of each shift, were analyzed for corticosterone, cortisol and alpha-amylase levels.
RESULTS: Cortisol levels were found to be higher in whole-night workers compared to day-time and half-night groups (P = 0.043). There was no difference between the groups in terms of corticosterone (p = 0.540) and alpha amylase levels (p = 0.864). There were no difference between the times of the shifts (beginning, middle, end) for cortisol and corticosterone levels but there was an interaction for salivary alpha-amylase levels (p=0.016).
CONCLUSION: Cortisol, the main glucocorticoid in humans, was higher in women working in whole-night shifts suggesting that these women have activated HPA axis even though the other trace glucocorticoid (namely corticosterone) did not differ. Moreover, different pattern of alpha-amylase secretion suggest that HPA and ANS systems work in different fashion in shift workers.
Acknowledgement: This study was supported by Inonu University BAP (Project # 2017/649).
Keywords: Shift system, cortisol, corticosterone, alpha-amylase, salivary, woman.

PC-16

Experimental Hypothyroidim Decreases Adropin, Asprosin and Preptin Levels in Male Rats

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AIM: Thyroid hormones have important roles on energy consumption through interactions with central and peripheral pathways. The aim of this study was to determine the effects of hypothyroidism on adropin, asprosin and preptin levels in rat.
METHODS: The study was performed on the 38 male Wistar rats. Experiment groups were designed as follows. 1-Control, 2-Hypothyroidism; To induce hypothyroidism Propylthiouracil (PTU) was applied by intraperitoneal as 10 mg/kg/day for 2 weeks. 3-Hypothyroidism + Thyroxine; Previously, hypothyrodism was induced by 1 weeks PTU application and then 1 week L-thyroxine was given by intraperitoneal as 1,5 mg/kg/day. After supplementation, the animals were sacrified and blood samples were collected for FT3, FT4, adropin, asprosin, preptin analysis.
RESULTS: FT3 ve FT4 levels were reduced significantly in hypothyroidism (P<0.001). Hypothyrodism resulted in decreased adropin, asprosin and preptin levels. However, thyroxine supplementaion after hypothyroidism corrected asropin and preptin levels (P<0.001).
CONCLUSION: The results of the present study show that experimental hypothyroidism leads to significant changes in blood adropin, asprosin and preptin levels. Thyroid function returned asprosin and preptin levels to their normal values following thyroxine supplementaion.
Keywords: Hypothyroidism, Preptin, Adropin, Asprosin.

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PC-17

The Role of Adropine and Irisin in an Experimental Hypertension Model

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AIM: Hypertension is an important public health problem with a high prevalence leading to cardiovascular deaths. Irisin, which is a myocin released from the skeletal muscle and it is separated from the FNDC5 protein. It is reported that adropine plays a role in energy metabolism by regulating insulin sensitivity and regulates endothelial functions. In our study, we aimed to determine the relationship of blood pressure with irisin and adropin levels in the experimental hypertension model and to investigate the role of these hormones in the pathophysiology of hypertension.
METHODS: In our study, 16 male Spraque-Dawley rats were divided into two groups as Control and Hypertension. In the hypertension group, 400 mg / L dose of Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME) was added to drinking water for 6 weeks. Blood pressure measurements by the tail-cuff plethysmography performed every week. At the end of the 6th week, after blood samples were taken under anesthesia, euthanasia was performed.
RESULTS: Compared with the control group, it was observed that the administration of L-NAME in the hypertension group increased the systolic and diastolic blood pressure significantly from the 2nd week ($p < 0.05$). It was observed that serum irisin levels decreased significantly in hypertension group compared to control, and the decrease in serum adropin levels was not statistically significant ($p < 0.05$).
CONCLUSION: Our findings showed that iris and adropine may play a role in the physiopathology of hypertension. We believe that further studies are needed to understand the mechanisms underlying this relationship.
This study was supported by TUBITAK 2209-A University Students Research Projects Support Program (1919B011800461).

Keywords: Adropin, Hypertension, Irisin, L-NAME.

PC-18

The Effect of Ebselen on Motor Performance, Balance Skills and Analgesia in A Rat Model of Parkinson's Disease

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AIM: Parkinson's Disease (PD) is a neurodegenerative disorder. There are many underlying mechanisms, which is associated with oxidative stress, neuroinflammation and apoptosis. Ebselen (EBS) is a non-toxic seleno-organic drug with antioxidant, antiinflammatory, and cytoprotective properties. We aimed to investigate the protective and therapeutic effects of EBS on rotenone (ROT)-induced rat model of PD.
METHODS: Sprague Dawley female rats were randomly divided into 4 groups (n=8). Control group rats were given only sunflower oil and DMSO: EtOH (2:1) as vehicle solution. ROT group: 3 mg/kg, once a day s.c. injection of ROT was applied for 7 days. EBS+ROT group: 10 mg/kg, once a day i.p. injection of EBS was applied for 7 days+3 mg/kg, once a day s.c. injection of ROT was applied for 7 days. ROT+EBS Group: 3 mg/kg ROT (s.c.) and 10 mg/kg EBS (i.p.) were injected for 7 days. All rats were tested for rotarod and accelerod balance-to-motor coordination performance measurements, hot plate and tail-flick analgesimetry tests, and the effects of EBS on the nervous system and functions.
RESULTS: There were no significant results among the groups in the hot plate and tail flick tests. At the 10, 20, 30 and 40 rpm test speeds, the Control and EBS+ROT group rats remained on the rotarod test longer than did the ROT group rats. The ROT+EBS group rats remained on the rotarod test longer than did the ROT group rats only 10 rpm test speed. Furthermore, in the 600 seconds accelerod test, in accordance with the rotarod test results the Control and EBS+ROT group rats remained for a longer duration than did the ROT group rats.

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CONCLUSION: These findings showed that EBS had protective effect on motor performance and balance skills in a rat model of PD.

Acknowledgement: This study was supported by TUBITAK (2209A Project).

Keywords: Ebselen, Parkinson's Disease, Rat, Rotenone.

PC-19

The Effect of Direct Electric Current on Motion Behaviour in a Transgenic β-Amyloid Model of Caneorhabditis elegans: An In-vivo Study

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AIM: Alzheimer's disease (AD) is a neurodegenerative disease associated with neuronal accumulation of β-amyloid (Aβ) protein resulting in neuronal dysfunction, cognitive disorders, dementitia, behavioural and personality changes. The aim of our study was to investigate the effects of direct electric current on AD and to diminish the prominent effects of dementitia symptoms in an in-vivo transgenic β-amyloid model of Caneorhabditis elegans (C.elegans). Aβ1-42 accumulates in an oligomeric form in body muscle cells of transgenic C.elegans leading to paralysis at 25°C within 24 hours.

METHODS: Transgenic C.elegans were maintained in Nematode Growth Medium placed in rectangular petri dishes plated with copper on two sides. Petri dishes were exposed to direct electric current for 10 minutes per one hour within periods. The study groups were classified as 1V Group, 1.5V Group and control group with no electric exposure. The direct electric current exposure was repeated until no alive C.elegans could be seen in petri dishes. Kaplan–Meier Survival Analysis was conducted for survival analysis.

RESULTS: The first paralysis was observed in 1V and 1.5V Groups between 3-6 hours periods. The most paralyzed C.elegans were around 16th hour periods. All C.elegans were paralyzed at 24th period in control group and and at 32nd period in 1.5V Group except the lost ones. One alive C.elegans was observed at 33nd period in 1V Group. The experiment was ended as all C.elegans were paralyzed in 1.5V and control groups. The survival estimation for 1V Group was higher than the other study groups (p=0.001).

CONCLUSION: Our results suggest that direct electric current exposure may prevent paralysis in transgenic β-amyloid model of C.elegans, regarding its genomic similarity to human genome. Further studies in human AD should be elucidated by means of direct electric current exposure.

Keywords: Alzheimer's disease, Direct electric current, Transgenic Caneorhabditis elegans, Paralysis.

PC-20

Effect of Naloxone Precipitated Morphine Withdrawal on Melatonin Receptor Expressions in Hippocampus and Hypothalamus Tissues in Morphine Dependent Rat

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AIM: Melatonin exerts many physiological effects via two different G protein-coupled membrane receptors, MT1 and MT2. Additionally, recent studies also reveal MT1/MT2 heteromer formation of these receptors. The aim of this study was to investigate gene expression levels of these melatonin receptors in morphine dependence and morphine withdrawal condition in rat hippocampus and hypothalamus.

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METHOD: Morphine sulphate was subcutaneously administered to adult male rat for 7 days at a dose of 10 mg/kg/day for morphine addiction model. Naloxone (1mg/kg) was injected to constitute morphine withdrawal condition to another group at 7th day of dependency. Naloxone induced morphine withdrawal symptoms were determined for 30 minutes and hippocampus and hypothalamus tissues were removed after decapitation of all rats. Melatonin receptors' gene expression levels were analysed by quantitative RT-PCR. One-way ANOVA was used for statistical evaluation.
RESULTS: Naloxone administration significantly increased wet-dog shakes compared to control and morphine dependent group ($p<0.05$). Teeth chattering numbers were also significantly higher in withdrawal group compared to dependent and control group ($p<0.001$). Mean jump number was 2.42±0.51 in naloxone group, while there was no jumping in the other groups. But, there was no difference in grooming behavior between withdrawal group and the others. As for RT-PCR results in hippocampus tissue, melatonin receptors' gene expression levels were not different in all groups. MT1 receptor gene expression was high in dependence and withdrawal group compared to control ($p<0.05$) in hypothalamus. Similarly, we determined elevated level of MT1/MT2 heteromer receptor formation of melatonin in addicted and withdrawal groups compared to control ($p<0.05$). MT2 receptor levels in hypothalamus were not different in all groups.
CONCLUSION: We specified naloxone-induced morphine withdrawal symptoms according to our results from the present study. Data also reveal that membrane receptors of melatonin may be involved in some pathophysiological processes in hypothalamic functions in morphine dependence and withdrawal situation.
Key word: Morphine dependency, naloxone, melatonin receptors, hypothalamus and hippocampus.

PC-21

The Effect of Exposure to Music on Spatial Learning and Memory in Rats

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AIM: The 'Mozart effect' is an enhancement in spatial learning and memory performance after listening to Mozart's Sonata for Two Pianos (K448). Increasing the number of NMDA receptors play a role in the Mozart effect. NMDA antagonists damaged the sensory-motor gating system and led deficits in the Pre-Pulse Inhibition (PPI) of the acoustic startle reflex. Aim of this study is to investigate Mozart effect on a spatial task in rats.
METHODS: Male Wistar rats were used. White Noise and White Noise+Ketamine groups were exposed to white noise, while Mozart and Mozart+Ketamine groups were exposed to Mozart's Two Pianos Sonata (K448) from postnatal day (PND)14. On PND 56, rats were trained in an 8-arm radial maze. Then, Pre-Pulse inhibition of the acoustic startle reflex was measured in White Noise and Mozart groups, and after 2,5 mg/kg intraperitoneal Ketamine administration to the WN+Ketamine and M+Ketamine groups.
RESULTS: Groups exposed to white noise made fewer working memory errors than groups exposed to Mozart's Sonata ($p<0.05$). There was no significant difference between reference memory error and total error measurements. Groups exposed to white noise were able to complete their tasks in a shorter time than groups exposed to Mozart's Sonata ($p<0.05$). The levels of PPI at 74, 78, 86 dB in white noise exposed groups are higher than groups exposed to Mozart's Sonata but not statistically significant. At 86 dB, PPI levels of the White Noise+Ketamine group was higher than Mozart+Ketamine group ($p<0.05$).
CONCLUSION: Our results show that Mozart's Sonata for Two Pianos (K448) does not enhance spatial learning and memory in rats.
Acknowledgement: This research was supported by Istanbul Medeniyet University Scientific Research Projects Unit (Project number: 1565).

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Keywords: Learning, Memory, Music, Pre-pulse Inhibition.

PC-22

Investigation of Age-Dependent Changes in Hippocampal Long-Term Potentiation Related Tau Phosphorylation

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AIM: Dementia is disruption of memory and other mental abilities with a severity that will affect daily life. 60-80% of diseases associated with dementia are Alzheimer's type dementia. Aging is an important factor in the formation of Alzheimer's disease, which is characterized by tau protein accumulation and tangle, especially in the cortex and hippocampus, since the majority of cases are 65 years old or older. The aim of this study is to investigate the effect of old age on hippocampal LTP and how the phosphorylation levels of different Tau epitopes are affected in stimulated hippocampus.
METHODS: The study was carried out on 2-month and 12-month old (n=10/group) Wistar albino male rats. Potential changes occurring in the hippocampal dentate gyrus region were recorded by applying high frequency stimulation to the performance pathway 4 times at 5-minute intervals to induce LTP. To evaluate LTP, EPSP slope and PS amplitude were measured by taking the average of the first 5 minutes after the LTP induction and in the last 5 minute time interval. For protein analysis, hippocampus in which LTP is induced was used. Protein analyzes were performed by western blot method.
RESULTS: The PS amplitudes decreased significantly with aging in the induction and maintenance periods without any change in the EPSP slopes ($p<0.05$). It was found in LTP-induced hippocampus that the total-Tau protein level and the p-TauThr181, p-TauThr231, p-TauSer199-Ser202, p-TauSer202-Thr205 epitopes were increased, while the p-TauSer416 epitope was decreased with aging.
CONCLUSION: Impaired LTP that occur with aging may be among the underlying causes of dementia that occurs in older ages. In addition, Tau epitopes known to play a role in the pathogenesis of Alzheimer's disease may support increased phosphorylation-impaired LTP responses with aging. These results may explain the causes of cognitive functions such as impaired learning and memory in old age. Supported by BAP (TCD-2016-6262).
Keywords: Hippocampus, Long-term potentiation, Tau phosphorylation.

PC-23

Investigation of Age-Dependent Changes in Hippocampal Long-Term Depression Related Tau Phosphorylation

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AIM: Dementia is disruption of memory and other mental abilities with a severity that will affect daily life. 60-80% of diseases associated with dementia are Alzheimer's type dementia. Aging is an important factor in the formation of Alzheimer's disease, which is characterized by tau protein accumulation and tangle, especially in the cortex and hippocampus, since the majority of cases are 65 years old or older. The aim of this study is to investigate the effect of old age on hippocampal LTD (Long term depression) and how the phosphorylation levels of different Tau epitopes are affected in stimulated hippocampus.
METHODS: The study was carried out on 2-month and 12-month old (n = 10/group) Wistar albino male rats. Potential changes occurring in the hippocampal dentate gyrus region were recorded by applying LFS (Low frequency stimulation) to the perforant pathway (1Hz,900 impuls,15 minutes) to induce LTD. To evaluate LTD, EPSP slope and PS amplitude were measured by taking the average of the first 5 minutes after the LTD induction and in the last 5 minute intervals. For protein analysis, hippocampus stimulated in electrophysiological studies were used. Protein analyzes were performed by western blot method.
RESULTS: For LTD, it was found that there was a significantly less suppression with aging in the induction period of EPSP and PS (population spike) ($p<0.05$), but no significant. It was found that the total-Tau protein level and the phosphorylation levels of p-TauThr231, p-TauSer396, p-TauSer416 epitopes were increased, while

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the p-TauSer202, Thr205 epitopes did not change with aging.
CONCLUSION: Impaired LTD that occur with aging may be among the underlying causes of dementia that occurs in older ages. In addition, Tau epitopes known to play a role in the pathogenesis of Alzheimer's disease may support increased phosphorylation-impaired LTD responses with aging. These results may explain the causes of cognitive functions such as impaired learning and memory in old age. Supported by BAP (TCD-2016-6262).
Keywords: Hippocampus, Long-term depression, Tau phosphorylation.

PC-24

Effects of Chronic High Fat Diet on Electrophysiological and Morphological Properties of POMC neurons in the Hypothalamic Arcuate Nucleus

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AIM: Obesity has become one of the most important health problems worldwide. The fundamental cause of obesity is the energy imbalance between calorie intake and expenditure. The hypothalamic arcuate nucleus (ARC) Proopiomelanocortin (POMC) neurons play an important role in energy balance and satiety feeling. For this purpose, POMC-Cre transgenic mice model was exposed to high-fat diet for different periods and then electrophysiological and morphological properties of POMC neurons were investigated.
METHODS: Adult transgenic POMC mice were used in this study. Adaptations of POMC-satiety neurons in mice models exposed to chronic high-fat diet was investigated by using cutting edge technological methods such as Cre recombinase dependent pharmacogenetics, electrophysiological (patch-clamp) and confocal microscopy methods. Student's t-test was used for statistical analyses.
RESULTS: POMC neurons send dense intrahypothalamic axonal projections and make synaptic connections to paraventricular nucleus (PVN) of hypothalamus. There was a significant decrease in axon projection density from the arcuate POMC neurons to PVN in the high-fat diet group compared to chow diet group ($p<0.01$). However, electrophysiological recordings showed that firing frequency and leptin sensitivity were similar in both groups.
CONCLUSION: These findings showed that high-fat diet for three months altered the axonal projections from ARC to PVN but were neither necessary nor sufficient to acutely change electrical properties and leptin sensitivity of POMC neurons in transgenic mice.
Acknowledgement: This study was supported by TUBITAK (Project no: 118S245).
Keywords: POMC, Arcuate Nucleus, Patch Clamp, Electrophysiology, High Fat Diet, Leptin.

PC-25

Chronic High-Fat Diet Impairs Gastric Motor Functions through Reduced Nitrergic Transmission in Rats

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AIM: Chronically consumed high-fat diet (HFD) has been shown to impair both autonomic vagal and enteric neurocircuitries in rodents so that it is considered as a risk factor for functional dyspepsia (FD). Delayed gastric emptying (GE) and reduced accommodation are commonly observed in FD patients. Nitric oxide (NO) plays a pivotal role in regulation of gastric accommodative relaxation and sphincter function which is one of the major determinants along with contractility. Recent studies reported the reduced number of nitric oxide synthase (nNOS)-positive neurons in rats fed HFD. Therefore, using in-vivo and in-vitro techniques, the present study was designed to investigate the HFD-induced alterations in gastric emptying, gastric smooth muscle contractility and nNOS expression in myenteric neurons.
METHODS: Male Sprague Dawley rats were fed with regular diet or a HFD (60% kcal by fat) from 4- to 16-week-old age. Throughout the diet period, body weight progression was monitored weekly. Solid GE was

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measured following an overnight fasting. In-vitro organ bath study was performed to assess contractile and relaxative responses of antral and fundic smooth muscle strips by applications of bethanechol (10-7-10−4 M) and sodium nitroprusside (SNP; 10-7-10−4 M), respectively. The expression of nNOS was quantified in longitudinal muscle-myenteric plexus (LMMP) whole mount preparations by immunohistochemistry.
RESULTS: Compared with control, HFD significantly ($p<0.01$) increased the BW, while delaying solid GE. Both in antrum and fundus of HFD rats, bethanechol-induced contractions and SNP-induced relaxations were reduced, significantly ($p<0.05$). HFD remarkably decreased ($p<0.05$) the number n-NOS-immunoreactive myenteric neurons in fundic and antral/pyloric regions.
CONCLUSION: The present data suggest that chronically exposure to HFD between early adolescence and adulthood results in obesity accompanied by dyspeptic symptoms and impaired gastric motor functions which appear to be mediated by reduced nitrergic neuromuscular transmission.
Keywords: High-fat diet, obesity, gastric emptying, nitric oxide, gastric motility.

PC-26

Chronic High-Fat Diet Affected Plasma and Hippocampal Corticosterone and Insulin Levels as well as Spatial Memory in Adult Male Rats: Endoplasmic Reticulum Stress Involvement

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AIM: This study was undertaken to investigate effects of chronic high-fat diet (HFD) on plasma and hippocampal insulin and corticosterone levels, the hippocampus insulin receptor amount, and spatial learning and memory with or without receiving 4-phenyl butyric acid (4-PBA) in male rats.
METHODS: Adult male Wistar rats were divided into high-fat and normal diet groups. Then each group was subdivided into dimethyl sulfoxide (DMSO) and 4-PBA groups. After weaning, the rats were fed with HFD for 20 weeks. Then, 4-PBA or DMSO were injected for 3 days. Subsequently, oral glucose tolerance test was done. On the following day, spatial memory tests were performed. Then the hippocampus insulin, corticosterone, and insulin receptor levels were determined.
RESULTS: HFD increased plasma glucose, leptin and corticosterone concentrations, hippocampus corticosterone content, food intake, abdominal fat weight and body weight along with impaired glucose tolerance. It decreased plasma insulin, and insulin content, and its receptor amount in hippocampus. HFD lengthened escape latency and shortened the duration spent in target zone. 4-PBA administration improved the HFD- induced adverse changes.
CONCLUSION: Chronic HFD possibly through the induction of endoplasmic reticulum stress and subsequent changes in the levels of hippocampal corticosterone, insulin and insulin receptor along with possible leptin resistance caused spatial learning and memory deficits.
Keywords: High fat diet, Endoplasmic reticulum stress, 4 PBA, Spatial memory, Insulin.

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PC-27

Effects of Short Term High-Fat Diet on Anxiety Behaviour in Male and Female Mice

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AIM: We have investigated effects of short term high-fat diet on anxiety behaviour in C57BL/6 mice of both genders.
METHODS: In this study, male (n=7) and female (n=8) C57BL/6 mice were fed by High-Fat Diet (HFD, 60%) for two months. Control animals (n=8) were fed with standard mice diet. At the end of two months, anxiety behaviour was assessed by using elevated plus-maze, light-dark box and open field test. For female animals, oestrous cycle was monitored, and only those female mice on diestrous stage were chosen for behavioural experiments. Three day interval was given as battery time between the behavioural tests. Student's t-test was used for statistical analysis.
RESULTS: In the elevated plus-maze, both male and female animals spent almost equal time in close and open arms. There was no significant difference between HFD and control groups in terms of gender. Distance covered by HFD animals was significantly higher than control animals in the elevated plus-maze for males ($p<0.05$). There were differences in walking distance parameter between female control and HFD groups, but these changes were not significant. Anxiety scores tested in the light-dark box test were not significantly different in the HFD group which spent more time in the dark area. Similar results were observed in the open field test and animals mostly spent their time in the outer area.
CONCLUSION: Our results show that short-term high fat diet does not significantly affect anxiety behaviour in male and female mice. It is thought that in the anxiety tests utilized in the present study, light might be the only factor influencing the animals as an aversive factor.
Keywords: Anxiety, Feeding, High Fat Diet.

PC-28

Hypothalamic Expressions of Apelin, Apelin Receptor and Neuritine in the First Generation Rat Pups: A Maternal Depression Model

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AIM: Hypothalamus has a critical role in food intake and metabolism. Maternal depression may cause some fetal and neonatal effects related to feeding. The present study was designed to investigate hypothalamic expression levels of apelin (AP), its receptor (APR) and neuritin in first generation pups of rat exposed to maternal depression and sertraline (an antidepressant) treatment.
METHODS: Chronic unpredictable mild stress protocols were applied to assess experimental depression model during gestation period. Pregnant rats were divided to control, sertraline (10 mg/kg), depression and depression+sertraline groups. Sertralin treatments were subcutaneously applied via osmotic minipumps for last 15 days of pregnancy. Hypothalamus tissues were dissected from the first generation pups at aging day 0, 30 and 150. mRNA level expression of AP, APR and neuritin were evaluated by using quantitative RT-PCR.
RESULTS: Hypothalamic APR and neuritin expressions were decreased with age when day 0 were compared with day 30 and day 150. However, AP expression was upregulated in the same days. Depression tends to downregulate but sertraline treatment increased expressions of AP and neuritin. When compared to the control and depression groups in day 0, both AP and neuritin expression were upregulated in sertraline group ($p<0.05$).

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Depression downregulated AP, APR and neuritin expressions ($p<0.05$) in day 150.
CONCLUSION: Our results suggest an evidence that maternal depression and sertraline administration may affect expressions of AP, APR and neuritin and thereby hypothalamic neurogenesis in the first generation pups. There is a need for further studies to investigate epigenetics mechanisms to understand pathophysiology of maternal depression.
Acknowledgement: This study was partially supported by TÜBİTAK (#215S616) and Necmettin Erbakan University Scientific Research Projects (#192018003).
Keywords: Hypothalamus, apelin, apelin receptor, neuritin, maternal depression, rat pup.

PC-29

Effects of Ileal Interposition on Plasma Glucagon Like Peptide (GLP-1) and Pancreas GLP-1 Receptor Expression in Rats with Metabolic Syndrome

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AIM: Metabolic syndrome is a cluster of metabolic abnormalities that include hypertension, abdominal obesity, insulin resistance, and atherogenic dyslipidemia. Our aim in this study was to ensure the remission of parameters that define metabolic syndrome with ileal interposition (IT), which is a surgical procedure, and to examine the effect of IT on plasma GLP-1 and pancreatic GLP-1 receptor expression.
METHODS: For metabolic syndrome; newborn male Wistar Albino rats were given monosodium glutamate (4 g/mg) on days 0, 2, 4, 6, 8, 10, and saline (0.01 ml/g) to the control group. At the 5th month, IT and sham operations were performed on rats with metabolic syndrome. For IT, the 15 cm intestine segment was provided with cuts from 10 and 25 cm regions prior to the ileocecal valve. The jejunum was cut 5 cm distal from Treitz's ligament and the 15 cm ileum was placed isoperistalticly in between. 2 months later; lipid levels, abdominal obesity, plasma insulin, glucose tolerance (OGTT), Lee index, insulin resistance (HOMA-IR), plasma GLP-1 and pancreas GLP-1R expression were evaluated.
RESULTS: In rats with metabolic syndrome, IT was found to significantly improve hyperinsulinemia ($p=0.02$), correct lipid profile (triglyceride $p=0.001$, total cholesterol $p=0.008$), normalize the Lee index ($p=0.01$) and insulin resistance. While IT did not affect the secretion of the incretin hormone GLP-1 from the L cells in the distal intestine, the pancreas increased the level of GLP-1R expression ($p=0.01$).
CONCLUSION: Based on the present results, it is suggested that the increase in pancreatic GLP-1R expression may play an important role in the improvement of metabolic syndrome criteria.
Acknowledgement: This study was supported by Pamukkale University Scientific Research Projects Coordination Unit (Project # 2017SABE016).
Keywords: Metbolic surgery, GLP-1, GLP-1R.

PC-30

A Comparison of Blood Factors, Cognitive Status, Moods and Mental Health Status, in Diabetic Patients Type 2 with Control Group

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AIM: This study was examined blood factors, cognitive status, moods and mental health in patients with type II diabetes in Diabetes Center of Shahid Mohammad Montazeri Hospital in Najafabad and compared them with a control group.
METHODS: In this cross sectional study, 49 patients with type II diabetes referring to Diabetes Center with average age of 51 years were compared with the control group with the average age of 50. Both groups were homogenized in gender and education. Biochemical parameters of blood, Hemoglobin A1C (HbA1c), Subjective Neurocognitive Inventory (SNI), Profile of Mood States (POMS), General Health Questionnaire (GHQ) in two

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groups were examined. For data analysis, Multivariate Statistical Analysis and Mann-Whitney Test have been done by software SPSS.
RESULTS: The diabetics group showed higher Fasting Blood Sugar (FBS), HbA1c than the control one ($p<0.05$). There were no significant differences between the two groups in other biochemical parameters. Diabetes group indicated lower psychomotor speed, non-verbal memory and initiated energy than the control group. Moreover result showed a significant increase in anxiety and physical illness and a significant decrease in Scale manure-friendly and Energy in diabetic group.
CONCLUSION: The findings showed not only levels of blood glucose and HbA1c in diabetic patients are very high, but also their mental health and mood status are lower than healthy group.
Keywords: Diabetes mellitus, Mental Health, A1C Hemoglobin.

PC-31

The Effect of High Fat or High Carbohydrate Diets Exposure During Gestation and Lactation Periods on Hypothalamic and Cerebellar Oxidative Stress Markers in Offspring Sprague Dawley Rats

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AIM: To determine the effect of exposure high carbohydrate and high fat during the maternal period on the enzymatic activity of glutathione-S-transferase (GST) and superoxide dismutase (SOD) and the levels of malondialdehyde (MDA) and glutathione S-reductase (GSH) in the hypothalamus and cerebellum of male offspring rats.
METHODS: Mother Sprague-Dawley rats were fed control (C), high-fat diet (HFD) and high carbohydrate diet (HCD) during the gestation and lactation (G-L) periods. After the lactation period (21day), a total of 18 male offspring rats from three groups were sacrificed. Enzymatic activities of SOD and GST and levels of GSH and MDA were measured in cerebellum and hypothalamus. Statistical analyses were performed by Kruskal Wallis and Man Whitney U Test. p values lower than 0.05 was accepted as statistically significant.
RESULTS: The enzymatic activity of GST was significantly higher in cerebellum and hypothalamus of HFD-exposed offsprings when compared to C-exposed offsprings ($p<0.05$). MDA level was significantly lower in the cerebellum of HCD exposed offsprings rats ($p<0.05$). There was an increasing tendency for MDA level in the hypothalamus of HCD exposed rats but this increase was not statistically significant.
CONCLUSION: Excessive carbohydrate and fat exposure during the maternal period have a modulatory effect on the oxidative stress status of different parts of the brain. These results indicate that increased GST activity through excessive fat exposure during G-L periods was an adaptive brain response. However, further molecular studies should be planned to clarify the associated mechanisms underlying the higher or lower levels of oxidative stress markers.
Keywords: Maternal diet, oxidative stress, hypothalamus, cerebellum, GST, MDA.

PC-32

Effects of Irisin on Reproductive Parameters in High Fat Diet Induced Obese Male Rats

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AIM: The effects of obesity on reproductive function in males has been increasingly recognized. However, there is almost no study investigating the effects of irisin, an adipomyokine, on the male rats' reproductive parameters. The present study was designed to investigate the possible effects of irisin on accessory sex organs and sperm parameters in the rat model of high-fat diet-induced obesity.
METHODS: For the study, a total of 40 male Sprague-Dawley rats were used. Animals were randomly divided into four groups: Control, irisin (subcutaneous irisin infusion (100 ng/kg) for 4 weeks), obese, obese+irisin (100 ng/kg s.c.) infusion for 4 weeks (n=10 for each group).

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Irisin and control groups were fed with a control diet. Obese and obese+irisin groups were fed with a high-fat diet to ensure diet-induced obesity. At the end of the study, the animals were decapitated by guillotine, and then left and right epididymis, testicular tissues, prostate, and seminal gland were taken and weighed respectively. Finally, sperm motility, morphology, and concentrations were determined. One-way ANOVA post hoc Tukey's HSD test was used for evaluation of the data. In all analyses, $p<0.05$ was considered statistically significant.
RESULTS: Irisin significantly decreased prostate weight in the only obese+irisin group compared with the obese group ($p<0.05$). Also, high-fat diet-induced obesity led to a significant decrease in testis, epididymis, and seminal vesicles relative weights' (mg/100g body weight) in obese and obese+irisin groups compared with the control group ($p<0.001$). The high-fat diet significantly decreased sperm motility ($p<0.05$) and increased head ($p<0.01$) and total abnormal sperm rate ($p<0.05$) in the obese group, and irisin significantly reversed all these effects in the obese+irisin group compared to the obese group.
CONCLUSION: Our results suggest that the chronic administration of irisin does not prevent high-fat diet-induced accessory sex organs' shrinkage, however, it may show the ameliorative effect on the certain sperm parameters in obese male rats.
Acknowledgment: This study was supported by TUBITAK (Project # 118S519).
Keywords: Obesity, irisin, accessory sex organ, sperm parameter.

PC-33

Effects of Different Types of Calorie Restriction on SIRT1 Levels in Mouse Brain

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AIM: Sirtuin 1 (SIRT1) is a NAD+-dependent deacetylase that maintain cell homeostasis by playing an important role in some cellular processes, such as gene transcription, life expectancy, stress reaction, immune system and apoptosis. Calorie restriction (CR) and other nutritional interventions modulate SIRT1 activity lead to increment in lifespan and metabolic rate in mammals. Also aging is the predominant risk factor for neurodegeneration and SIRT1 is positively influencing the preservation of neurological processes that deteriorate during aging. In this research, we report a biological link between sirtuin1 activity and effects of different types of calorie restrictions and their role on aging brain in mice.
METHODS: To investigate SIRT1 expression due to aging and calorie restriction, we used female C57BL/6 mice at 10 and 49/50 weeks of age and from four different dietary groups. Enzyme-linked immunosorbent assay was employed to examine the concentration of SIRT1 from brain homogenates and explore the underlying mechanisms of chronic calorie restriction (CCR) and intermittent calorie restriction (ICR) in relation to aging at the proteomic level. RESULTS: In the current study, mice applied CCR and ICR had higher SIRT1 concentration when compared to ad libitum (AL) group mice. In addition, 10-weeks-old mice had the highest SIRT1 protein level.
CONCLUSION: Our results suggest that SIRT1 relative concentration in the brain is reduced with aging regardless of the type of calorie restriction.
Keywords: NAD-dependent deacetylase sirtuin-1, calorie restriction, aging.

PC-34

Central Autonomic Pathways Contribute to Delayed Colonic Transit Induced by Peripheral Apelin-13

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AIM: Peripherally administered apelin-13 has been demonstrated to inhibit colonic transit (CT) through cholecystokinin (CCK)-mediated pathway in rodents, however, the relevant action is incompletely understood. Besides the gastrointestinal (GI) tract, APJ receptor is expressed in circumventricular organs (CVOs) which have dense neuronal projections to the brainstem structures regulating autonomic signaling. Moreover,

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peripheral exogenous apelin was demonstrated to induce c-Fos expression in CVOs. Therefore, the present study was designed to investigate the involvement of central autonomic pathways in delayed CT induced by peripheral administration of apelin-13.

METHODS: A silicon catheter was inserted via the cecum into the proximal colon and fixed with sutures in adult male Wistar rats. Then, chemical sympathectomy was utilized by 6-hydroxydopamine (6-OHDA) and/or parasympathetic innervation of colon was eliminated by subdiaphragmatic vagotomy (VGX) plus pelvic denervation (PD). Following a 7-day recovery, CT was spectrophotometrically quantified as the geometric center of distribution of phenol red solution applied through the catheter. Apelin-13 (100 µg, ip) was injected 90 min prior to CT measurements. APJ receptor and c-Fos expression was detected in frozen brainstem sections by immunofluorescence staining.

RESULTS: Compared to control rats, apelin-13 significantly ($p<0.01$) delayed CT. The delayed CT was partially attenuated ($p<0.05$) by VGX+PD, while more pronounced ($p<0.01$) restoration was observed in rats underwent chemical sympathectomy. No additional effect was observed when both sympathectomy and parasympathectomy were applied. Apelin-13 administration remarkably induced c-Fos immunoreactivity in area postrema cells that express APJ receptor.

CONCLUSION: The present data suggest that autonomic network, predominantly the sympathetic pathway is involved in inhibitory action of peripheral apelin-13 on colon motility which appears to be mediated through area postrema.

Keywords: Apelin, colon motility, sympathetic, parasympathetic, area postrema.

PC-35

Expression of Melatonin 1 Receptor in Patients with Gastric Adenocarcinoma and Its Relationship with Clinicopathological Features

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AIM: Gastric cancer accounts 8% of the total cancer cases leading to 10% of total cancer deaths worldwide. The indoleamine N-acetyl-5-methoxytryptamine, better known as melatonin, is the principal hormone produced by the pineal gland. Recently, it has been well documented some anti-cancer roles of melatonin in some malignancies as breast and colon cancer; as well as some its protective roles in the GI tract that have been known as free radical scavenger, antimitogenic and apoptotic properties. According to the anti-cancer effects of melatonin, wide distribution of this neurohormone in GI tract and some proposed physiologic and pharmacologic roles for this neurohormone and following our previous study which has shown expression of MT2 receptor in gastric adenocarcinoma, this study initially scheduled to determine the expression of melatonin receptor MT1 in tissue samples of adenocarcinoma cancer patients.

METHODS: A total of 10 gastric adenocarcinoma patients and 10 normal individuals were examined for MT1 gene expression by real-time PCR. Additionally, for screening of different alleles of MT1 in our samples, the SSCP-PCR procedure was developed.

RESULTS: Our results have shown interestingly high expression for MT1 receptor in cancer and marginal cancer groups comparing with normal group. Our findings also have shown that a remarkable association between MT1 receptor mRNA levels and grade in individuals over age 50. PCR-SSCP analysis results showed a variation between individuals which may be effective on their gene expression patterns.

CONCLUSION: According to our knowledge, for the first time this study evaluated the expression of MT1 receptor gene in gastric adenocarcinoma tissues which consistent with our previous study but with some difference in comparisons between kind of tissue expression and difference in polymorphisms. Moreover, these results show the defending role of melatonin in the GI system.

Keywords: Melatonin, gastric adenocarcinoma, MT1 receptor, gene expression, polymorphism.

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PC-36

Melatonin may Regulate Apoptotic Pathway via Affecting Bax, Bcl2I1 and XIAP Levels in Myocardial Ischemia-Reperfusion Injury

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AIM: Restoring blood flow after ischemia is very important for maintaining the viability of ischemic tissue. The reperfusion of ischemic tissue causes enzyme degradation, excessive increase in reactive oxygen species and secondary injuries such as apoptosis. Melatonin released from the pineal gland is one of the important endogenous antioxidants. The aim of this study was to investigate the effect of melatonin on proapoptotic B-cell lymphoma 2 associated X (Bax) and antiapoptotic B-cell lymphoma 2I1 (Bcl2I1) and X-linked inhibitor of apoptosis (XIAP) levels in ischemia/reperfusion (I/R) injury in rat heart.
METHODS: Rats were randomly divided into 3 groups as control, I/R, and I/R+melatonin. The left main coronary artery was occluded for 30 min followed by 120 min reperfusion. Melatonin was administrated by intraperitoneal injection during the last 10 day. Bax, Bcl2I1 and XIAP expression levels were analyzed by real time-PCR.
RESULTS: The tissue Bax and MDA levels were increased (1,47 and 3,82 fold, respectively) while Bcl2I1 and XIAP levels were decreased with I/R injury. Melatonin administration showed protection against I/R induced myocardial injury by inhibiting all these changes.
CONCLUSION: Antiapoptotic XIAP and Bcl2I1 and proapoptotic Bax may be involved in signaling pathways in the pathology of myocardial I/R, and the protective role of melatonin may be due to its antiapoptotic activities.

Keywords: Melatonin, ischemia-reperfusion injury, apoptosis, Bax, Bcl2I1, XIAP.

PC-37

Effects of Melatonin and Postconditioning on Reperfusion Arrhythmias and Nesfatin-1 Levels

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AIM: Reperfusion arrhythmias are considered as an indicator of injury to the cardiac conductive cells during restoring blood flow to the ischemic heart. Nesfatin-1 is an adipokine that inhibits the inflammatory response. The inflammatory process has been reported to be an important factor in cardiac rhythm disorders. The aim of this study was to investigate the effect of ischemic postconditioning (PostC), a cardiac protective phenomenon, and melatonin, an antioxidant hormone, on reperfusion arrhythmias and nesfatin-1 levels.
METHODS: Rats were randomly divided into 4 groups as control, ischemia-reperfusion (I/R), I/R+PostC and I/R+melatonin. The left coronary artery was occluded for 7 min and reperfused for 7 min. At the beginning of reperfusion, PostC was applied as three cycles as ischemia for 10 sec and reperfusion for 10 sec and melatonin was administrated by intraperitoneal injection during the last 10 days. Arrhythmias were evaluated as appropriate in Lambeth Conventions. The nesfatin-1 level was analyzed by the ELISA method.
RESULTS: Ventricular extrasystole and incidence of ventricular tachycardia due to I/R injury decreased with PostC and melatonin treatment, and the time to first ventricular extrasystole prolonged with treatments. Nesfatin-1 levels decreased significantly with I / R, while it increased significantly with PostC and melatonin.
CONCLUSION: PostC and melatonin may reduce reperfusion arrhythmias. The decrease in nesfatin-1 levels may be one of the factors causing the arrhythmias. PostC with melatonin may have similar protective effects on reperfusion arrhythmias and nesfatin-1 levels.

Keywords: Reperfusion arrhythmias, postconditioning, melatonin, nesfatin-1.

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PC-38

Estimation of the Factors Associated with Diabetes Mellitus by Multilayer Perceptron Artificial Neural Network Model

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AIM: Diabetes mellitus (DM) is a chronic and endocrine disease characterized by high levels of blood glucose (BG), which causes harmful effects on the heart, blood vessels, eyes, kidneys, nervous system, etc. Around the world, nearly 1.6 million people died from DM which is seventh leading cause of the death in 2016. In the past three decades, the prevalence of DM has risen dramatically, and approximately 422 million people have DM worldwide. Identification of factors associated with DM is essential for the management of DM. This study aimed to estimate the factors associated with DM by multilayer perceptron (MLP) artificial neural networks (ANNs).
METHODS: Open access DM data of this study was taken from the website (www.kaggle.com/saurabh00007/diabetescsv#diabetes.csv). The target/output variable is the presence or absence of DM, and the input/estimator variables are selected as body mass index (BMI), BG (mg/dL), diabetes pedigree function (DPF), blood pressure (BP; mmHg), age, pregnancies, insulin (mIU/L), and skin thickness (ST; mm). The MLP ANNs were used to estimate the factors associated with DM. The performance of the model was determined by the accuracy, cross entropy error and area under receiver characteristic curve.
RESULTS: 69.3% (532) of data were used in training and 30.7% (236) of data were used in testing procedures. The accuracy rates of DM for the designed model were calculated as 79.1% in the training dataset and 80.9% in the testing dataset. Importance values of the factors were determined as 0.244 for BMI, 0.233 for BG, 0.132 for DPF, 0.128 for BP, 0.082 for age, 0.074 for pregnancies, 0.057 for insulin and 0.050 for ST.
CONCLUSION: According to the results, the three most important factors associated with DM were BMI, BG and DPF. The results of the proposed model in this study can be used in preventive medicine and management of factors associated with DM.

Keywords: Artificial neural networks, Diabetes mellitus, Estimation, Multilayer perceptron, Risk factors.

PC-39

Effects of Systemic Administration of Cisplatin and Hydrogen Sulfide on Cerebrum and Cerebellum Tissue in Rats

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AIM: We have previously shown that cisplatin (CIS) causes nephrotoxicity, hepatotoxicity and autotoxicity by oxidative damage and inflammatory pathway at doses of 5 mg/kg and 16 mg/kg in rats, respectively. In this study, we aimed to investigate the effects of systemic administration of CIS (7,5 mg/kg) and a combination of CIS with hydrogen sulfide (H2S, 10 µmol/kg), an endogenous gasotransmitter that exerts antioxidant and anti-inflammatory properties, on cerebrum and cerebellum.
METHODS: Thirty-two rats were divided into four groups as follows; Control, CIS (7,5 mg/kg intraperitoneal (ip) single-dose CIS), H2S+CIS (10 µmol/kg ip NaHS for 7 days and 7,5 mg/kg single dose ip CIS on 4th day) and CIS+H2S (4 days after 7.5 mg/kg single dose ip CIS administration 10 µmol/kg ip NaHS for 7 days). Histopathological and biochemical analyses of cerebrum and cerebellum were carried out.
RESULTS: There was a slight decrease in the number of intact neurons in the CIS group when compared to the control group, and a slight increase in CIS+H2S group when compared to the CIS group. CIS group showed an increase in glutathione peroxidase activity in the

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cerebrum and a slight decrease in superoxide dismutase (SOD) activity in the cerebellum when compared to the control group. H2S+CIS group showed an increase in MDA production in the cerebrum and a decrease in SOD activity in the cerebellum when compared to the control and CIS groups. CIS+H2S group pointed an increase in MDA production in the cerebrum and a decrease in SOD activity in the cerebellum when compared to the control group.
CONCLUSION: Systemic administration of CIS at a dose of 7,5 mg/kg did not cause significant cerebrum damage in the rats. H2S administration induced a further increase in lipid peroxidation in the cerebrum and exacerbated a deficiency of antioxidant defense caused by CIS in the cerebellum.
Keywords: Cisplatin, Hydrogen sulfide, Cerebrum, Cerebellum, Antioxidant, Neurotoxicity.

PC-40

Effects of Ramelteon on Myometrial Contractions in Rats

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AIM: Ramelteon is a melatonin MT1/MT2 receptor agonist, which is used especially in the treatment of sleep disturbance. Melatonin has previously been known to have an inhibitory effect on uterine contractions. However, although the use of ramelteon during pregnancy in category C. There is no study showing whether it has any activity on uterine contractions. In this study, we aimed to investigate the effects of ramelteon on uterine contractions.
METHODS: Sprague-Dawley female rats were used in the study (n=7). Longitudinal myometrium strips with dimensions of 12mmx2mmx1mm were prepared for examination in isolated organ bath. The strips were placed in an isolated organ bath by applying 1 g of tension and isometric contractions were recorded. The tissues were left to equilibrate for 90 min with rinsing every 30 min and to achieve spontaneous contractions. Following the regulation of spontaneous contractions, 0.5 µM and 1 µM doses of the ramelteon were administered as a double dose at 60 min intervals and monitored for 30 minutes. Statistical analyses were performed using the Paired Sample t-Test.
RESULTS: Ramelteon decreases the frequency value by 83.1%, peak to peak (p-p) value by 90% and amplitude value by 97% after the application of 0.5 µM dose. In ramelteon treatment at 1 µM dose, it decreased the frequency value by 90%, p-p value by 97% and area value by 100% ($p<0.001$). Thus, melatonin-like inhibitory activity on uterine contractions were determined.
CONCLUSION: It is thought that ramelteon, which is used in the treatment of sleep disorder and can be used in pregnancy, shows its inhibitory activity on uterine contractions over MT1/MT2 receptors such as melatonin. Ramelteon may be recommended for use during pregnancy, taking into account its inhibitory effect on uterine contractions.
Keywords: Ramelteon, contraction, myometrium, rat.

PC-41

Effetcs of Levetiresetam on Contraction of Myometrium in Rats

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AIM: Levetiracetam is a second generation antiepileptic drug. Levetiracetam inhibits the release of calcium from intracellular stores in the neuronal cell. Although the pregnancy category is in C, its effectiveness on uterine contractility has not been studied before. In our study, we aimed to investigate the possible efficacy of levetiracetam on uterine contractile activity in non-pregnant rats.
METHODS: Rats were used in Sprague-Dawley intact diestrus period. Uteruses were prepared and placed in an isolated tissue bath. Spontaneous contractions were expected to decrease. Then, at 60 min intervals, 20 µM and 40 µM of levetiracetam was applied to isolated organ bath chambers. It was followed for 30 minutes. Ca-free Krebs solution was applied to determine the cellular mechanism by which the change in uterine activity occurred. Then, spontaneous contractions were waited to

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end. After spontaneous contractions ended, 40 µM of levetiracetam was applied to isolated organ bath chambers. The area, peak-to-peak (p-p) and frequency values of uterine contractions were analyzed before and after administration of levetiresetam. The data obtained from the analysis were evaluated using the Paired T-Test in the SPSS 21.0 Statistics Software.
RESULT: After levetiracetam spontaneous contractions decreased, there was a statistically significant increase in p-p, area and frequency of contractions after administration of 20 µM and 40 µM doses ($p < 0.05$). The increase in p-p, area, frequency values after application at 20 µM dose was higher compared to 40 µM dose ($p<0,05$). The same application was performed with the Ca free solution we used to explain the cellular mechanism of this increase. However, the same activity was not observed.
CONCLUSION: As a result of our study, it was shown that levetiracetam application has an activator effect on uterine contractions. It is thought that this activity of levetiracetam is by increasing extracellular calcium passage into the cell.
Keywords: Levetirasetam, myometrium, contraction, rat.

PC-42

Melatonin Administration Increases the Expression of Sirtuin-1 and PGC-1α in Heart Tissue of Rats with Aged-Diabetic

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AIM: Silent information regulator 1 (SIRT1) is a conserved NAD+ dependent histone deacetylase and has been implicated in a variety of intracellular signals, such as senescence. Previous studies have demonstrated that activation of SIRT1 improved the cardiac dysfunction. Therefore, SIRT1 has been regarded as a target for cardiac dysfunction. Recently, there is some evidence that melatonin confers protective effects against cardiac dysfunction. Besides, melatonin has been reported to be a potent regulator of SIRT1 in many diseases. The aim of this study was to investigate how melatonin administration in aged female rats with diabetes affects the levels of heart tissue SIRT1 and PGC-1alpha.
METHODS: The study was performed on aged female rats (16 months old) who were provided by The Experimental Medicine Research and Application Center of Selçuk University. The study protocol was approved by the Ethics Committee of Experimental Animals of Experimental Medicine Research and Application Center of Selçuk University. A total of 24 aged female rats were divided into 4 groups: Group 1. Control, Group 2. Melatonin, Group 3. Diabetes, Group 4. Diabetes + Melatonin. Diabetes was induced by intraperitoneally with 40 mg/kg streptozotocin (STZ). Melatonin was supplemented as 5 mg/kg/day by IP for 4 weeks. At the end of the study animals were sacrified under general anesthesia, heart tissue samples obtained and SIRT1 and PGC-1α protein gene expression were determined by PCR.
RESULTS: The highest heart tissue SIRT1 and PGC-1α expression values were obtained in the diabetes+melatonin group ($p<0.05$) and the lowest heart tissue SIRT1 and PGC-1α levels were determined in the diabetes group ($p<0.05$).
CONCLUSION: The results of present study indicated that melatonin administration increased the expression of SIRT1 and PGC-1α in heart tissue of aged diabetic rats. Chronic melatonin therapy can cure the reduction in SIRT1 and PGC-1α expression caused by diabetes in the aged rat model.
Keywords: SIRT1, melatonin, PGC-1α, diabetes, heart tissue, elderly female rats.

PC-43

Melatonin Administration Increases on the Expression of GLUT 4 in Heart Tissue of Rats with Aged-Diabetic

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AIM: Melatonin has been shown to modulate GLUT4 expression in cell culture medium. It was also reported that decreased GLUT4 expression in pinealectomized rats reached normal levels with melatonin

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supplementation. Reporting of reduced GLUT4 expression in heart tissue of hyperthyroid rats treated with melatonin supplementation also indicates an inevitable association between melatonin and cardiac GLUT4 expression. The purpose of this study was to investigate how melatonin supplementation affects GLUT 4 levels in heart tissue in an aged female rat model with diabetes induced by streptozotocin.
METHODS: The study was performed on aged female rats (16 months old) at the Experimental Medicine Research and Application Center of Selçuk University. The study protocol was approved by the Ethics Committee of Experimental Animals of Experimental Medicine Research and Application Center of Selçuk University. A total of 24 aged female rats were divided into 4 groups: Group 1. Control, Group 2. Melatonin, Group 3. Diabetes, Group 4. Diabetes + Melatonin. Diabetes was induced by intraperitoneally (IP) with 40 mg/kg streptozotocin (STZ). Melatonin was supplemented as 5 mg/kg/day by IP for 4 weeks. At the end of the study animals were sacrified under general anesthesia, heart tissue samples obtained and GLUT 4 protein gene expression were determined by PCR.
RESULTS: The lowest heart tissue GLUT4 levels were determined in the diabetes group (G3), ($p<0.05$). However, melatonin supplementation normalized decreased cardiac GLUT 4 expression in the diabetic rats.
CONCLUSION: The results of present study indicated that melatonin administration is increases the expression of GLUT 4 in heart tissue of aged diabetic rats. Chronic melatonin therapy may prevent the reduction in GLUT 4 expression caused by diabetes in the aged rat model.
Keywords: GLUT4, melatonin, diabetes, heart tissue, aged female rats.

PC-44

Melatonin Inhibits Oxidative Stress in Heart Tissue in Aged Diabetic Female Rats

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AIM: Cardiomyocytes are particularly sensitive to oxidative damage due to the link between mitochondria and sarcoplasmic reticulum necessary for calcium flux and contraction. Melatonin is the main product of the pineal gland and has antioxidant and anti-inflammatory activity and cardioprotective properties. On the other hand, metabolic disorders in diabetes mellitus lead to more severe inflammation and overproduction of free radicals. Therefore, diabetes mellitus is a main risk factor for coronary heart disease. The aim of this study was to investigate the effects of melatonin administration on heart tissue damage of a diabetic and aged female rat model.
METHODS: The study was performed on female aged rats (16 months old) which were provided by The Experimental Medicine Research and Application Center of Selçuk University. The study protocol was approved by the local ethics committee. A total of 24 aged female rats were divided into 4 groups: Group 1. Control, Group 2. Melatonin, Group 3. Diabetes, Group 4. Diabetes + Melatonin. In order to produce experimental diabetes, streptozotocin (STZ) was injected as 40 mg/kg by intraperitoneal in groups 3 and 4. Animals were sacrificed under general anesthesia at the end of 4 weeks experimental period, and heart tissue samples were taken and examined for MDA ve GSH by ELISA.
RESULTS: The highest heart MDA values were found in the diabetic group (G3; $p<0.05$). MDA values of the diabetes + melatonin group (G4) were significantly lower than all the other groups ($p<0.05$). The highest heart GSH values were found in the diabetes + melatonin group (G4) and the lowest heart GSH levels were found in the diabetes group (G3, $p<0.05$).
CONCLUSION: The results of our study showed that oxidative stress in heart tissue can be prevented by melatonin supplementation in diabetic aged female rats.
Keywords: Melatonin, oxidative stress, diabetes, heart tissue, aged female rats.

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PC-45

Regulatory Effect of Melatonin Supplement on Cardiac Contraction Parameters in Diabetic Aged Female Rat Model

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AIM: It has been shown that melatonin as main product of the pineal gland, exerts anti-diabetic properties through regulating various cellular mechanisms. However, the effects of melatonin on the cardiac muscle are controversial. The aim of this study was to investigate the effects of melatonin supplementation on myocardial papillary muscle contraction parameters in a diabetic elderly female rat model.
METHODS: The study was performed on female aged rats (16 months old) at the Experimental Medicine Research and Application Center of Selçuk University. The study protocol was approved by the Ethics Committee of Experimental Medicine Research and Application Center of Selçuk University. A total of 24 elderly female rats were divided into 4 groups: Group1; Control, Group 2; Melatonin, Group 3; Diabetes, Group 4; Diabetes+Melatonin. Diabetes was induced by intraperitoneally with 40 mg/kg streptozotocin. Melatonin was supplemented as 5 mg/kg/day by IP for 4 weeks. At the end of the experiment, for the electrophysiological recordings in the isolated organ bath, the hearts of the animals were quickly removed under anesthesia and the left ventricular papillary muscles were isolated in the solution adjusted to pH 7.4. As a result, the contraction force and durations of the papillary muscles of all animals were measured in an isolated organ bath.
RESULTS: In our study, the responses of the myocardial papillary muscles of diabetic rats to stimuli at frequencies of 0.2, 0.5, 1, 2, 3, 4 and 5 Hz in the isolated organ bath were evaluated. At all frequencies of stimulation, the contraction force of the animals decreased significantly, while the contraction duration was significantly prolonged ($p<0.05$). Melatonin supplementation significantly increased the animals' contractile strength ($p<0.05$), but did not significantly affect the duration of contraction.
CONCLUSION: The findings of our study show that the reduction in myocardial papillary muscle contraction force can be restored with melatonin supplementation in a diabetic elderly female rat model.
Keywords: Cardiac contraction, melatonin, aged female rat, diabetes.

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