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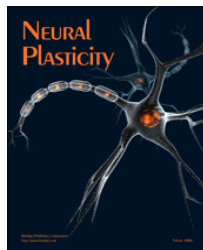
Proceedings:

1st International ISBS Summer School on Behavioral Genetics and Neuroscience of Stress

St-Petersburg, Russia



May 9-15, 2008



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Welcoming Address from the School Chair

Dear friends,

I would like to welcome all the participants of the 1st ISBS Summer School on behavioral genetics and neuroscience of stress, to be held in St. Petersburg, Russia, in May 2008. Promising young scientists from almost 15 different countries will be participating in this year's curriculum, and we all are looking forward to this educational experience.

The School has a multidisciplinary program, integrating the recent developments from a variety of specializations in the field. It has been designed to foster an environment of interactive scientific exchange, where these new issues of both experimental and theoretical aspects of neuroscience will be discussed. A particular focus will also be given to new concepts (that are offering valuable perspectives on neuropsychiatric disorders) and new methods for experimentation.

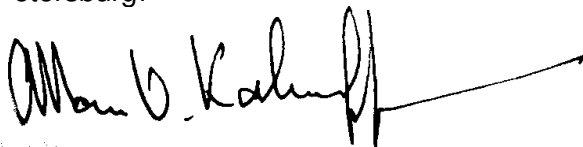
As you all know, the investigation of brain disorders has long been preoccupied with studying one disorder at a time, in hopes that exploring that single domain will allow for a better dissection of neurobehavioral and neurogenetic correlates. However, in the clinical field, it is becoming increasingly understood that brain disorders are often occurring in "spectra", rather than in isolation.

This calls into question the efficacy of testing single domains in animal experimental models, and suggests that the complexity of human phenotypes should be applied to the field of animal modeling. Such approach will allow for a much more clinically relevant examination of the disorders, as they will represent a far more accurate portrayal of the pathogenetic phenomena present in patients.

Using a new set of conceptual tools, researchers can begin to see the larger scope of the pathogenetic networks and establish solid genetic, behavioral, and endocrinological links between the disorders. This strategy, needed to match the goals of basic research and clinical treatment, will be among key topics of this School.

In addition to the substantial topical coverage, another strength of this School is that it will be held adjacent to the 11th International "Stress and Behavior" Conference, which brings together delegates from nearly 40 countries around the globe. This will present the opportunity for the students to attend both the school and the subsequent conference, discuss important topics with leaders in the field, and establish valuable collegial collaborations.

We are confident that our Summer School will offer its participants an interesting, professionally enriching experience in a perfect environment – the beautiful city of St. Petersburg!

A handwritten signature in black ink, reading "Allan V. Kalueff". The signature is written in a cursive style with a long horizontal line extending to the right.

Allan V. Kalueff, PhD Hon

School Abstracts

PRELIMINARY EXPLORATORY RESEARCH ASSESSING BRAIN ELECTRICAL ACTIVITY BEFORE AND AFTER INITIAL APP (AUDIO-PSYCHO-PHONOLOGY) THERAPY SESSIONS

SC van Hoorn, JJ Vervoort, W van den Bergh, J van Deun, W Linn
APP Institute, Sint Truiden, Belgium

Introduction: Audio-Psycho-Phonology (APP) was developed by the late Professor A Tomatis, a French ENT Physician in the 1950's. The therapy uses high filtered frequencies of sonic stimuli with auditory stimulation, and indications for the therapy include a range of neurological and psychological/psychiatric diagnoses. At the therapy centre and clinic in Sint Truiden, Belgium, research using quantitative EEG's and Brain Mappings, was started in 2005, relating to the use of the therapy particularly for learning disabilities and developmental delay in children. The research was undertaken to assess the changes in brain functioning, resulting from the therapy, which could correlate with the clinical results. Methods: Resting EEG's with and without Auditory Evoked Potentials were taken of a group of over 50 children (during the first 4 months of research), one day before APP therapy was started, and again 10 days later, at the end of the initial phase of intensive therapy. The EEG's were taken under standardised conditions. For each patient, 21 electrodes were used to create quantitative EEG data. This data was then converted into 3-dimensional Brain Mappings using the FFT (Fast Fourier Transform) method looking at the Delta, Theta, Alpha and Beta activity, and assessed along with normal values from the BEAM (Brain Electrical Activity Mapping) database. Results and discussion: Definite positive changes in brain activity were seen on the Brain Mappings after the therapy. Briefly, in overview, areas of abnormal under-activity especially in the temporal and frontal regions appeared to normalise, and N1, N2 and P300 values also improved. Research is still continuing and up to date; well over 500 Brain Mappings have been made. Further analyses of the Brain Mappings are in process. Correlating specific brain activity changes to changes/improvement of clinical symptoms and signs is also in process. Conclusion: As yet, no final concrete conclusions have been made. However, obvious positive changes in brain electrical activity, seen in the brain mappings, result after APP therapy.

THE DOSE-DEPENDENT EFFECT OF 5HT_{2C}-RECEPTOR AGONIST MK 212 ON MICE BEHAVIOR

MA Tibeikina, EG Kuznitsova, TG Amstislavskaya, NK Popova
Institute Cytology and Genetics SB RAS, Novosibirsk, Russia

Brain serotonin (5-HT) is involved in numerous physiological events. 5-HT affects various CNS functions: sleep, learning and memory, pain and different types of behavior, such as: aggressive, sex, feeding, neuroendocrine regulation, motor activity and biological rhythms. 5-HT is thought to play a significant role in various types of pathological conditions in humans, namely anxiety, aggressiveness, panic, schizophrenia, autism, etc. Recently, at least 14 subtypes of 5-HT receptors have been reported. It is important to investigate how the definite types and subtypes of serotonin receptors can influence different kinds of behavior. The aim of study was to explore the effect of 5HT_{2C}-receptor selective agonist MK-212 in several doses on anxiety, acoustic startle reflex, motor activity, body temperature and corticosterone level in mice. The experiment was carried out on 320 male CBA /Lac mice weighing 23-25 g.

In three days before testing mice were isolated into individual cages. MK-212 was dissolved in sterile saline and injected intraperitoneally (0.1, 0.2, 0.5, 1.0 mg/kg) in 25 min before testing. Control animals were injected saline. Intact animals were the additional control. Methods: 1) anxiety was studied using the light/dark box (LDB) test. The time which a mouse spent in the light compartment of the box during the test period (5 min) related to decreased anxiety. 2) to investigate the startle reflex and the prepulse inhibition animals 4 pulse and 4 prepulse + pulse alternated with approximately 15-20-s rest intervals were given 3) the number of crossed squares (horizontal activity), rearings (vertical activity), grooming, the number of entrance into central area and the time in the center (indices of anxiety) were recorded in the open-field test during 5 min. 4) tail suspension test was used to reveal the body temperature reaction on the stress (suspension). At first the initial body temperature was detected. Then the mice were suspended by the tail for 5 min, and body temperature was measured at 15 and 30 min from the beginning of the test with a digital thermometer. Animal behavior was videotaped and analyzed with a computer program Ethostudio. 5) to investigate stress response, a blood corticosterone level was measured. The males were decapitated after tests. The method of concurrent protein binding was used. Results: Injection of MK 212 intraperitoneally in high doses (0.5 and 1.0 mg/kg) had no effect on the anxiety in the DLB. The time which a mouse spend in the light compartment of the box during the test period related to decreased anxiety. In low doses of 0.1 and 0.2 mg/kg the agonist reduced anxiety. It was hypothesized that low doses of MK 212 exhibited an anxiolytic effect in mice. 2) The injection of 5HT_{2C}-receptor agonist MK 212 in all doses tested (0.1, 0.2, 0.5, 1.0 mg/kg) resulted in increasing of the startle amplitude compared with control and intact animals ($p < 0.05$). Injection of only the highest dose of MK 212 (1.0 mg/kg) significantly increased the prepulse inhibition in mice ($p < 0.05$ compared with the control). 3) In the open field test, the injection of MK 212 in high doses (0.5 and 1.0 mg/kg) reduced motor activity in mice. In low doses of 0.1 and 0.2 mg/kg the agonist had no effect on motor activity. 4) A significant increase of body temperature in all groups of mice was shown after the tail suspension test ($p < 0.01$ compared with the initial temperature). The increase of body temperature after injection of high doses of MK 212 (0.5 and 1.0 mg/kg) was less than after the low doses (0.1 and 0.2 mg/kg). 5) Injection of MK 212 in high doses (0.5 and 1.0 mg/kg) augmented blood level of corticosterone ($p < 0.05$ compared with the control and intact animals). In low doses, (0.1 and 0.2 mg/kg) the agonist had no effect on blood level of corticosterone. To sum up: the dose-dependent effect of 5HT_{2C}- receptor agonist MK 212 on mouse behavior was demonstrated. Intraperitoneal injection of MK212 in high doses (0.5 and 1.0 mg/kg) increased blood level of corticosterone in mice and reduced their locomotor activity. In low doses (0.1 and 0.2 mg/kg), the agonist reduced anxiety but had no effect on locomotor activity or corticosterone level. The increase of body temperature after injection of MK 212 was less than after the lower doses (0.1 and 0.2 mg/kg). It is hypothesized that low doses of this compound exhibited anxiolytic activity in mice. The agonist augmented the startle amplitude in all doses used. The data obtained expand our knowledge about the mechanisms of behavioral regulation by serotonin and hypothalamo-pituitary-adrenal axis and the role of 5HT_{2C} receptor subtype in these processes.

GENETIC STRUCTURE OF HEREDITARY CATALEPSY IN MICE

DV Bazovkina, EM Kondaurova, AV Kulikov, NK Popova
Institute of Cytology and Genetics, SB RAS, Novosibirsk, Russia

Catalepsy or pronounced freezing is a natural passive defense strategy in animals and a syndrome of some mental disorders in humans. Hereditary catalepsy was shown to be associated with depressive-like features in rats and mice. Mice of the antidepressant-sensitive catalepsy (ASC) line selectively bred for high predisposition to catalepsy from the

(CBAx(CBAxAKR)) backcross population between catalepsy-prone CBA and catalepsy-resistant AKR strains showed depressive-like behavioral alterations. Moreover, catalepsy in ASC mice was sensitive to chronic but not acute treatment of imipramine. We have mapped by QTL-analysis the major gene of catalepsy on the 47- to 75-cM fragment of mouse chromosome 13. However, QTL analysis methods are developed for mapping quantitative traits and they are hardly applied to binary traits. The main aim was to map the genes encoding predisposition to catalepsy in mice using congenic lines and selective breeding experiments. Methods: The mapping of the major gene of catalepsy on chromosome 13 was accomplished by comparison of the catalepsy percentage in the congenic lines AKR.CBA-D13Mit76C, AKR.CBA-D13Mit76A and AKR.CBA-D13Mit78 carrying the 59- to 70-, 61- to 70- and 71- to 75-cM fragments of chromosome 13 transferred from the CBA to the AKR genome. Catalepsy is estimated by measuring the time during which an animal maintained an immobile posture on parallel bars (with the forepaws at a 45° angle above the hind legs). The 46 males representing the 46 different families of ASC mice were genotyped with a genomewide set of 51 polymorphic microsatellites in order to assess the effect of selective breeding on the distribution of CBA and AKR alleles. This effect was evaluated as the deviation of the allele concentrations from the expected 3:1 ratio (like in the backcross population) using χ^2 test and Bonferroni correction for false positives. Results and discussion: Catalepsy was found only in the AKR.CBA-D13Mit76C (52%) and AKR.CBA-D13Mit76A (52%) mice. So the major gene of catalepsy was mapped on the fragment of 61–70 cM which contains 34 protein-coding genes but only 8 are highly expressed in mouse brain: Gbbp1 (vasculin), Map3k1 (microtubule-associated protein), Rpl41 (ribosomal protein L41), Il6st (gp130 signal transducer), Ppap2a (phosphatidic acid phosphatase 2a), Gzmk (granzyme K), Snag1 (sorting nexin-associated golgi protein 1) and Hspb3 (heat shock protein 3). These putative candidate genes regulate general intracellular functions, such as signal transduction and apoptosis. At the same time, no gene regulating the function of specific neurotransmitters involved in the mechanisms of freezing was detected in the 61- to 70-cM fragment of chromosome 13 linked to catalepsy. Selective breeding of the (CBAx(CBAxAKR)) backcross generation for high predisposition to catalepsy showed 26 genome-wide distributed CBA-derived alleles on chromosomes 1, 3, 4, 5, 6, 7, 8, 9, 11, 13, 14, 15, 18 and 19 as well as 9 AKR-derived alleles mapped on chromosomes 10, 12, 16, 17, 18 and 19 that increased the cataleptogenic effect of the major gene. Conclusion: Consequently, the genetic structure of hereditary catalepsy in mice includes one major gene mapped on the fragment of 61–70 cM on chromosome 13 and 31 genome-wide distributed genes-modifiers. Although the minor genes-modifiers were unable to induce catalepsy along, they increased significantly the cataleptogenic effect of the CBA alleles of the major gene. Since hereditary catalepsy in mice is associated with depressive-like state and sensitivity to antidepressant drugs, the mapping of the loci defining predisposition to catalepsy seems to be important for understanding the genetic mechanisms of depression.

THE BEHAVIORAL NEUROPHYSIOLOGY OF ACUTE STRESS: EFFECTS ON PLACE CELL ACTIVITY IN THE SUBICULUM

J Passecker, A Della Chiesa, V Hok, SM O'Mara

Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland

Introduction: Stress has a deleterious impact on the brain, body and behavior. Here we investigate how stress impacts on information processing in the hippocampal formation, a brain structure particularly sensitive to stress, and implicated in memory and spatial representation. We record from hippocampal formation neurons in freely-moving animals, using subicular place cells as a model system to quantify how stress impacts on neuronal information processing. Methods: Young Wistar rats (200-300g) were implanted with a 16 channel microdrive array (custom built). After a seven day recovery period, habituation trials

commenced (20min/day/5 days) in an open field box (dimensions: 80x80cm). Place cells were recorded (Axona, UK) at three different time points: Prior to the induction of stress, 30 minutes after stress and the last measurement was performed 4hrs after stress induction. Stress was induced by exposing the rats individually to a brightly-lit elevated box in a separate room. At the end of the experiment rats were euthanized and brains perfused and stained for electrode position verification. Fecal samples were collected throughout the experiment at designated time points for measurement of corticosterone levels. Data analysis was completed using Tint (Axona, UK) and other customized analysis software. Results and Discussion: Preliminary data suggests a size reduction of the place field after stress induction. Moreover, fewer spikes discharge in the non-place field areas of the maze suggesting a sharpening of the firing characteristic. Further, a trend towards rate remapping is observed which means firing location remains constant whereas the firing rate is changed. Conclusion: The experiments presented here show the direct examination of the effects of behavioral stress on hippocampal spatial representation and spatial processing *in vivo*. To fully understand the underlying mechanisms regarding the effects of stress on place cells it is necessary to determine cellular factors at play and seek ways to ameliorate any such deficits.

EVALUATION OF THE ROLE OF MONOAMINES IN ELECTROCONVULSIVE THERAPY IN AN ANIMAL MODEL OF PARKINSON'S DISEASE

AM Landau, A Nahimi, AK Olsen, G Wegener, P Videbech, A Gjedde, D Doudet

PET Center and Center of Functionally Integrative Neuroscience, Århus University, and Institute for Basic Psychiatric Research, Department of Biological Psychiatry, Århus University Hospital, Århus, Denmark

Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder in which the dopaminergic neurons in the substantia nigra degenerate. Depression is one of the most disabling co-morbidities associated with PD and both PD and depression are poorly treated in advanced patients. The development of alternative, safe, and effective treatments for PD motor symptoms and depression is an important area of research. A routine treatment for depression, electroconvulsive therapy (ECT), has additional beneficial effects on the motor symptoms of PD. Little is known about the mechanisms of action of ECT in PD patients. Rodent studies do not allow long term longitudinal studies of regional changes in monoamine neurotransmission and ethical concerns greatly reduce the feasibility of performing extensive longitudinal studies in human PD subjects. Research plan: We propose to investigate the effect of ECT-induced widespread brain stimulation on the monoaminergic systems involved in both PD and depression in a neurotoxin-induced minipig model of PD. Furthermore, we will determine if ECT can induce regionally specific neurotrophic factor expression in the normal and lesioned nigrostriatal systems and limbic structures. 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) will be administered to minipigs to create a bilateral model of PD with rigidity, bradykinesia, swallowing dysfunction and gait disturbances. Behavioral rating with a standardized motor scale developed for pig studies will be performed in normal and parkinsonian pigs before and after a clinical course of ECT (3 times per week; 10 treatments; Thymatron stimulator) under thiopental anesthesia. Twelve untreated and 12 MPTP-lesioned pigs (9 active ECT and 3 sham in each condition) will be positron emission tomography (PET) scanned with an HRRT PET scanner before and after the end of the ECT/sham course (48 hrs, 1 week, 4 weeks and 3 months). At each time point, pigs will be sacrificed and the brain taken out. One hemisphere will be used to assess neurotrophic factor expression; the other will be used for autoradiographic binding of monoaminergic receptors and transporters to determine the nature of changes in dopamine and serotonin neurotransmission induced by ECT. This will provide the rationale and pilot data for the design of studies concerning the effects of ECT in human subjects. Conclusion: This study will be the first comprehensive

longitudinal study of the mechanisms of action of a non-pharmacological intervention with known therapeutic effects in both PD and depression. The use of electrical currents to treat various neurological and psychiatric conditions is an active field of research currently lacking well characterized models with which to investigate the mechanism of therapeutic benefits. Validation of the minipig as an animal model will be a valuable contribution to the field. Better understanding of the mechanisms of action of ECT may help in the design of improved therapeutic strategies for depression and/or PD.

THE ASSOCIATION GENE POLIYMORPHISM OF RENIN ANGIOTENSIN SYSTEM AND BEHAVIOR

VA Shleptsova, MA Kulikova, AG Tonevitsky

Faculties of Basic Medicine and Biology, Moscow State University, Russian Research Institute of Sport and Physical Education, Moscow, Russia

Introduction: The brain RAS has been found to be involved in the modulation of cardiovascular and fluid-electrolyte homeostasis. The RAS has additionally been implicated in other brain-specific functions, such as memory, cognition and stress. The physiologically active octapeptide angiotensin II in the brain, mediated by angiotensin receptor type 1 (AT1) and type 2 (AT2), involve modulations in neuronal activity. It is well-known that neuronal AT1 receptors mediate the stimulatory actions of Ang II on blood pressure, water and salt intake, and secretion of vasopressin but the function of AT2 receptors remains controversial. There are a few studies about influence of AT2 receptor on personality. Methods: In the current investigation, 160 healthy persons (male=78; female=82) participated. Their mean age was 19±2 years. The individual level of aggression was assessed with Buss-Durkee Hostility Inventory (BDHI). Personality traits were studied with 16 factories R. Kettell scale, Eysenck Personality Inventory, and the Five-Factor Nonverbal Personality Questionnaire. Venous blood was collected from each subject, and genomic DNA was isolated. Determination of gene polymorphism C3123A of AT2 receptor gene was performed using the polymerase chain reaction (PCR). The results of PCR were documented with gel electrophoresis. Statistical calculations were performed using the program "STATISTICA" for Windows (StatSoft Inc., USA). Results and discussion: We found an association between the AT2 receptor gene polymorphism and any type of aggression: assault (physical aggression) ($p=0.04$), verbal aggression ($p=0.05$), negativism ($p=0.03$). Interestingly, those heterozygotes have a lower level of aggression than homozygotes. They are not as impulsive as the homozygotes. It is very intriguing that heterozygotes are more anxious ($p=0.03$; $p=0.02$) and have a higher level of emotional liability ($p=0.05$, $p=0.01$). The cause of this effect is that some RAS components can affect neurotransmitters (norepinephrine, dopamine, serotonin), which influence behavioral characteristics. Conclusion: In our investigation, we studied the relationship between gene polymorphisms of the AT2 receptor and personality characteristics that indicate an influence of renin angiotensin system components on neurotransmitter signaling.

INTEGRATING BEHAVIORAL PHENOTYPING TECHNIQUES AND NEW DOMAIN-ORIENTED CONCEPTS IN BIOLOGICAL PSYCHIATRY

JL LaPorte, AV Kalueff

National Institute of Mental Health, NIH, Bethesda, MD, USA

Introduction: Biological psychiatry and biopsychology are important fields that can offer many valuable insights into the neural substrates of brain disorders. Our research interests have been focused on elucidating the mechanisms of these disorders through various rodent models, particularly using behavioral neurophenotyping techniques. In addition, we attempt to integrate new recently-developed concepts into these investigations in order to promote

effective translational research. Methods: Our research has utilized different tools for high density data acquisition/analysis such as SigmaScan Pro and Noldus Ethovision. Also, experimental techniques such as the rodent forced swim test, elevated plus maze, open field test, Suok test, social interaction test, and chronic stress (rat exposure to mice) are being used in these investigations. Results and discussion: We have applied both single-domain and hybrid experimental methods in conjunction with the domain-interplay concept (Kalueff et al., 2008) to reveal behavioral neurophenotypes in serotonin transporter (SERT) mutant mice on two different genetic backgrounds. These behavioral and pharmacological studies have contributed to a more full characterization of the genetic animal models with reduced serotonin transporter function, and may add to our understanding of this transporter's role in normal and abnormal brain mechanisms. Conclusion: Our first goal is to employ new concepts in the field of behavioral neuroscientific research. Incorporating hybrid models and the concept of domain interplay is part of the effort to enhance the translational validity of neuropsychiatric research. By using this new knowledge, we can reach our second goal of contributing to the understanding of brain disorders and finding inventive ways they may be treated. This research was supported in part by the Intramural Program of the NIH, NIMH and by a NARSAD YI Award.

THE RELATIONSHIP BETWEEN EXECUTIVE ATTENTION AND POLYMORPHISM 2756C/T MGLUR8 IN MALE

JV Shchegolkova

Moscow State University, Moscow, Russia

The glutamatergic signaling pathway represents a candidate susceptibility system involved in the mechanism of forming attention and working capacity. One attractive candidate for studying the molecular physiology of attention is the glutamatergic receptor subtype 8 (mGluR8). Expression of mGluR8 was observed in the olfactory system, the neocortex, and the limbic cortex (including the hippocampus and the amygdala). Using electron microscopes, mGluR8 was largely observed on the axon terminals. Especially in several regions of the hippocampus, it was found in the active zone of both asymmetrical and symmetrical synapses where mGluR8 may regulate glutamate release as an autoreceptor or GABA release heterosynaptically. The influence of mGluR8 polymorphism located 29 bp after the termination codon (2756C/T) on executive attention was conducted on volunteers. 108 students of Moscow State University (mean age 20 ± 2 , girls = 61, boys = 47) were tested by Schulte tables. We found a significant association of mGluR8 polymorphism with executive attention only in males. Carriers of T allele (TT,CT) implemented the test faster than CC group ($p=0,02$).

EFFECT OF GENES FROM THE SEROTONIN SYSTEM ON THE MENTAL FATIGUE

MA Timofeeva, MA Kulikova, NV Maluchenko, AG Tonevitsky

Biological Faculty, Department of Bioengineering, Moscow State University, Moscow, Russia

Introduction: Fatigue is an important factor affecting sporting and mental performance. It is complicated process which includes peripheral and central components. Central fatigue occurs in central nervous system as a result of mental as well as physical activity. There are evidences that serotonin is implicated in central fatigue development. Serotonin turnover in the brain increases in response to physical exercise. Decreased motivation, tiredness, loss of motor coordination - markers of central fatigue, are associated with the rise of brain serotonin concentration. But the role of serotonin in the development of mental fatigue is unclear. To explore the role of serotonin system in mental fatigue investigation of the impact of genetically

driven variation in serotonin function could be helpful. Methods: In the current study we investigate associations of serotonin system genes polymorphism with the mental fatigue. In the study students of Moscow State University took part (N=120, male=57, female=63). Volunteers were exposed to intensive mental workload (logical tasks and monotonous persistent completion of psychological tests) during 3 hours. Before and after workload the psychophysiological state was estimated with the help of "NS-Psychotest" (Neurosoft, Russia). Additionally volunteers completed self-ratings of mental fatigue and of functional state. Results and discussion: Serotonin activity in the brain is regulated by a transporter located in the plasma membrane of the cell (5HTT), which returns serotonin to the cell for recycling or metabolic degradation. Thus 5HTT plays an essential role in determining the duration and intensity of the serotonin communication with its receptors. Serotonin receptor 2A is a widely distributed postsynaptic receptor. It was detected that carriers of both ss-genotype of the 5HTTLPR (serotonin transporter linked polymorphic region) and CC-genotype of the 5-HT2A polymorphism (T102C) reported higher level of mental fatigue after performing fatigue task compared with carries of ll (5HTTLPR) and TT(T102C) genotype. ss-genotype of the 5-HTTLPR as well as CC-genotype of the 5-HT2A are associated with reduced expression of both genes and therefore could cause increasing concentrations of extracellular serotonin in the brain. These findings suggest a role of serotonin transmission efficiency in mental fatigue.

EFFECT OF POLYMORPHISMS IN THE DOPAMINERGIC SYSTEM GENES ON THE MENTAL FATIGUE

MA Kulikova, MA Timofeeva, NA Maluchenko, AG Tonevitsky
Moscow State University, Biological Faculty, Moscow, Russia

Introduction: Mental fatigue (MF) represents a failure to initiate and sustain tasks that require self-motivation and internal cues in the absence of demonstrable cognitive failure or motor weakness. So development of the mental fatigue is a significant problem for people who work with a large amount of data and need focused attention during the entire working day, as they are constantly in a stressful situation. The molecular mechanisms of MF origin are still not clear. However, it is known that the basal ganglia plays a key role in the development of MF. The basal ganglia appears to be capable of concurrent participation in a number of separate functions, such as motor, cognitive and limbic processing, due to the parallel structure of the individual basal ganglion circuitry. Dopamine is one of the most prevalent neurotransmitters in the basal ganglia. Therefore, we suppose that alterations in the functioning of the dopamine system provided by genetic variations may influence the development of MF. We tested the hypothesis that allelic variation of the DRD2 and DAT genes, located mostly in the striatum, could be associated with differences in the onset of MF. Methods: In the current investigation, 120 volunteers (57 males and 63 females) participated. All subjects gave informed consent after the nature of the experiment was explained to them. Subjects were genotyped for TaqI A RFLP of the DRD2 gene and 40-bp VNTR polymorphism of the DAT gene. Psychophysiological indexes such as level of attention, visual-motor reaction, and power of tapping were measured before and after mental load, which consisted of monotonous personality questionnaires during three hours. Also, self-ratings of mental fatigue were assessed before and after mental load. Results and discussion: All the analyses were made in the program Statistica 6.0. Associations between genotypes of DRD2 and DAT polymorphisms and self-ratings of mental fatigue were determined with Analysis of Covariance (ANCOVAs) with the genotype as the independent categorical factor. Case-control analyses suggested a strong association between the 9A1+ genotype and increased mental fatigue ($P < 0.05$). Also we showed that carriers of 9A1+ genotype had an increased

score of introversion and decreased striving for leadership. In conclusion, mental fatigue seems to be related to allelic variations within the DRD2 and DAT genes.

BEHAVIORAL CHANGES AND ALTERATION OF SKIN CONDUCTANCE IN RATS AFTER EXPOSURE TO CHRONIC SOCIAL STRESS

VM Lozova, OA Kovalenko, II Tubaltceva, DV Gorlov, VB Bogdanov, M Ju Makarcuk
Kyiv National Taras Shevchenko University, Kyiv, Ukraine

Introduction: Social stress is widely distributed through the population and plays a crucial role in numerous illnesses. Thus, it is important to investigate stress-induced and stress-related alterations in an organism, including behavioral changes. On the other hand, numerous experiments show the necessity of finding effective indicators of stressed state of the organism. Indicators such as skin electrodermal activity can be used to detect conditions of sympathetic division of vegetative nervous system. The aim of this study was to investigate stress-induced behavior of rats and to use parameters of electrodermal activity as an indicator of the condition of an organism. **Methods:** The behavioral procedure of social defeat consisted of five daily conditioning sessions that involved the same pairs of residents and intruders. The 45 min conditioning sessions started at 10:00 A.M. They were divided into two consecutive periods. During period I (30 min), intruders were placed singly inside the resident home cage, but were separated from them by the protective grate that allowed unrestricted visual, auditory, and olfactory contacts with the resident, but precluded close physical contact. During period II (15 min), the protective grate was removed with the resident present, allowing physical confrontation with the intruder. After the fifth conditioning session (i.e., on the sixth day), intruders and control rats were tested in the Elevated plus maze (EPM). On the second and third days after last stress exposure rats were tested in Open field test and Suok test, accordingly, to investigate the stress-induced behavior. After all the behavioral testing was done, the electrodermal activities of rat's palm were measured. On the same day the skin potentials were measured, the weights of rat's adrenal gland were evaluated. **Results and discussion:** Decreased locomotor and exploratory activity was shown in the Open field test and Suok test in stressed group. However, behavioral activity in control group of rats did not show such alterations. In the EMP, the stressed group of rats spent significantly less time in open arms compared with non- stressed rats. This data suggest that 5-days chronic stress exposure results in more stress-related behavioral patterns. The adrenal gland weight was more, on average, in stressed rats (20%) compared to control animals. They also registered significantly lower skin potential response frequency compared to control rats. In our experiments, we demonstrated that lower SPRF correlates with a lower level of locomotor activity in Suok test, indicating a higher level of anxiety. **Conclusion:** Our data suggest that chronic social stress results in a higher level of noophobic behavior and behavioral alterations, such as elevated numbers of escapable behavioral acts. On the other hand, in our experiment we showed that measuring electrodermal activity can be a good indicator of stress.

INTERACTION BETWEEN BEHAVIORAL PARAMETERS AND SKIN POTENTIALS IN ADULT RATS

OA Kovalenko, VM Lozova, DS Gorlov
Kyiv National Taras Shevchenko University, Kyiv, Ukraine

Introduction: The aim of this study was to discover the relationship between the level of skin potentials and individual parameters of behavioral activity of rats (intensity of locomotor and exploratory activity, processes of arousal and inhibition, emotionality). Our investigation can help to describe functional states of organisms and, in the future, in human beings by

analyzing parameters of electrodermal activity of the skin. Methods: This investigation was done on white male rats. The level of behavioral activity was measured in such behavioral tests as the Open field test, Suok test, Elevated plus maze (EPM). Also, electrodermal activity was measured from the left hind leg of the rat and indifferent electrodes at the bottom of the tail. Skin potential level (SPL), skin potential response frequency (SPRF), and skin potential response amplitude (SPRA) was registered in this method. In the behavioral tests, we registered locomotor activity, exploratory activity (horizontal and vertical), grooming (number and frequency), and time spent in open and closed arms in EPM. All behavioral tests were 5 min in duration and skin potentials were measured during a 10 min period. Results and discussion: A positive correlation was shown between behavioral activity and electrodermal activity of rats. The level of SPRF correlates with parameters of values of grooming (emotional activity and anxiety). SPL and SPRA correlates with the number of crossed segments in the Suok test (locomotor activity) and also the value of SPRA concerns the time spent in open arms of the EPM (indicating the level of anxiety). Because electrical potentials admittedly depend on sudoriferous gland activity and the level of sympathetic nervous system activation, electrodermal activity may represent functional activity of the organism on the system level. Various emotional states may cause the different physiological answers that affect potential values. That is why fluctuations of skin potentials may suggest a different level of activation in rats (may be used as a stress test) and correlate with individual behavioral parameters such as level of anxiety and emotional activity. Conclusion: Thus, the method of measuring electrodermal activity gives us the possibility to appreciate the functional state of organism on the more system-defined level, this is a methodological approach that gives allows us to take more system results and decrease the variables. Electrodermal potentials are used in clinical research for diagnosing the state of the patient. In our investigation, we showed that potentials may be used to evaluate the individual differences in behavioral activity and changes in its level after different influences.

THE FEATURES OF DIFFERENT ADAPTIVE BEHAVIORAL PATTERNS FORMING
SV Utevska, AM Titkova
Institute of Neurology Psychiatry and Narcology of AMS of Ukraine, Kharkiv, Ukraine

Stress situations require the coordinated efforts of all parts of an organism's adaptive systems. In spite of the universality of these systems of adaptation, however, the behavioral patterns have a wide variability and sometimes include compromising behavior, neurotic reactions or aggressive behavior. Low serotonin levels modulate these frequently aggressive acts through high impulsiveness. Possibly, this aggressive behavior is a response to the chronically stressful situation. It seems to be probable that more stress-resistant compromising behavior is based on the strong stress-limiting system with high serotonin level. Aggressive acts take place in situations when compromising behavior is not enough for solving the problem. Possibly, for animals with a weak stress-resistant system, the aggressiveness is the compensatory mechanism which is forming during the constant expectation of a stressful situation, and which is preventive toward expected or, possibly, modulated conflict. Apparently, the features of adaptive systems functioning can affect learning processes in the period of adaptation to stressful situations, but the opposite influence is not excluded. Therefore, our question is: what roles do the mechanisms of learning play in relation to the system of adaptation, under stressed conditions, and what role do the genetic determinants play in these processes? Our group is currently working on finding answers to these questions.

EXPLORATORY BEHAVIOUR AND POSITIVE EXPRESSIVE EMOTIONALITY: TWO STABLE BEHAVIOURAL PHENOTYPES IN LABORATORY RATS

D Matrov

Institute of Psychology, University of Tartu, Estonia

I am interested in studying the behavioral phenotypes in rats that can be identified with resilience towards stressful life events. I hypothesize that the high levels of expression of a particular behavioral disposition have a protective effect against adverse life events while low levels make animals more vulnerable to chronic stress regimen. One of the behavioral phenotypes is exploratory behavior; hereby I briefly summarize the main findings so far. Rats with high (HE) and low (LE) exploration levels in the exploration box were submitted to a 5 week long chronic variable stress (CVS) regime, during which they were weekly tested for sucrose 1% solution intake and preference and in several behavioral tests after CVS was ended. Stressed animals of both LE and HE groups had a slower weight gain. The LE stress group animals showed less sucrose solution intake and preference in comparison to the LE control group animals during CVS. Exploratory activity of the HE stress animals was unchanged after 5 weeks of CVS while the HE control group animals showed a decrease in their activity levels. Chronic stress increased the time of social interaction significantly with a previously unknown partner while control animals had very low baseline activity levels in the test. In conclusion, CVS regime was effective in producing the anhedonic effect and showed a complex interaction pattern with animal baseline exploration activity levels in several behavioral tests. To reveal brain regions most significantly related to individual differences in exploratory behavior, oxidative metabolism was measured by cytochrome c oxidase histochemistry in 2 months old Wistar rats with persistently high or low exploratory activity in a novel environment. LE-rats had significantly higher levels of oxidative metabolism in dorsal raphe and inferior colliculi. In contrast, HE-rats had higher metabolic activity in entorhinal cortex. In conclusion, rats with different exploratory styles differ in underlying cerebral activity as measured via oxidative metabolism in regions implicated in defensive behaviors and cognitive processing of sensory stimuli. Another behavioral phenotype is expressive positive emotionality. Manipulation of juvenile rats in a way that mimics the rough-and-tumble play and resembles tickling elicits 50-kHz ultrasonic vocalizations (USVs) that have been proposed as a measure of positive affect. In the present experiments the stability of the 50-kHz USV response (chirping) over 1.5 months of daily manipulation and the effect of tickling was studied. By the second week of tickling rats of both sexes developed a level of 50-kHz USVs that remained individually characteristic. During tickling the rats also emitted low levels of 22-kHz USVs. No correlation was found between the two types of USVs. In tests used in anxiety and depression research, tickling on its own had an anxiolytic effect in many experimental settings. Rats which expressed high level of chirping (HC-rats) were similar to low-chirping (LC) rats in anxiety measures but had lower activity in an exploration test and lower sucrose preference. LC-rats adopted more active coping strategies in the forced swimming test. These findings suggest that there are individually characteristic 50-kHz USV response levels to tickling in rats, and that HC- and LC-rats are similar with regard to anxiety levels but have different coping strategies to novelty.

SEARCH FOR BRAIN REGIONS INVOLVED IN SOCIAL BEHAVIOR AND STRESS RESPONSE

M Kanarik

Institute of Psychology, University of Tartu, Estonia

Social behavior is an aspect in human life that can be disturbed in pathological conditions such as depression, schizophrenia, anxiety disorders and autism. The neurobiology of

sociability relies greatly on monoamine neurotransmitters serotonin (5-HT) and dopamine (DA). Stress is a tool for humans and other animals to adapt to changes in environment, but prolonged periods of stress can often have counter-adaptive effects on behavior. The aim of the study was to identify the brain regions mediating 1) response to chronic stress combined with partial serotonergic denervation and 2) social behavior, and 3) to determine the extracellular monoamine levels in one of the regions revealed by metabolic mapping of the neuroanatomical substrate of sociability. Animals were tested in the social interaction (SI) test three times and divided into groups with high, medium and low sociability trait (HS, MS, LS). Chronic variable stress (CVS) was carried out for three weeks, parachloroamphetamine (PCA) (2 mg/kg) was injected a week before CVS. Brain regions involved in the behaviors assessed were determined with cytochrome oxidase (CO) histochemistry - an indicator of long-term energy metabolism. Extracellular levels of DA and 5-HT were assayed using microdialysis, HPLC and electrochemical detection. In the microdialysis experiment, a social stimulus, another rat separated by a grid, was presented to the test animal. Monoamine release was induced with parachloroamphetamine (PCA, 2 mg/kg) to assess release potential. In dorsomedial caudate putamen LS animals had higher CO activity than HS animals. In median preoptic nucleus, posterior paraventricular thalamic nucleus and median raphe the relationship between sociability and oxidative metabolism was nonlinear: MS-rats had higher CO activity than LS- and HS-animals. In supraoptic nucleus the results were complex – in the anterior part there was a linear relationship, with HS-rats having higher CO activity than LS-animals, but in the posterior part the results were non-linear, with MS-rats having lower CO activity than LS- and HS-animals. DA release increased in caudate putamen in response to social stimulation irrespective of the animal's sociability. 5-HT levels decreased irrespective of social stimulation in both groups, but the decrease was more pronounced in HS-rats. PCA treatment induced a massive increase in both DA and 5-HT release, but only 5-HT levels depended on sociability – in HS-rats, compared to LS-animals, more 5-HT was released in response to PCA. PCA induced 5-HT release correlated positively with the mean time spent in social interaction in three SI tests. PCA pretreatment blocked the increase in oxidative activity in stressed rats in medial preoptic area, cortical and medial amygdala. CVS reduced the oxidative activity induced by PCA in suprachiasmatic hypothalamus, anteroventral and dorsomedial part of ventrolateral thalamus, hippocampal CA3 region and cortical amygdala. In the dorsal part of the anterior olfactory nucleus stress blocked the decrease in oxidative activity evoked by PCA. In anterior paraventricular nucleus stress and PCA combined yielded in a reduction of metabolic activity compared to stress group. In substantia nigra PCA raised oxidative activity compared to control animals. There are differences in brain energy metabolism between animals differing in sociability trait levels in a number of brain regions. Social contact can induce DA release and highly social rats seem to have higher 5-HT release potential in the caudate putamen. PCA and CVS have independent effects on CO activity, both manipulations tending to increase it. In several regions PCA and CVS had an interactive impact on energy metabolism, the combination of manipulations resulting in oxidative energy metabolism comparable to control animals.

THE EFFECT OF PSYCHOSOCIAL STRESS PRE-ENCODING VERSUS PRE-RETRIEVAL OF HIPPOCAMPALLY-BASED EPISODIC MEMORY

JL Moore, RAP Roche

National University of Ireland, Maynooth, Trinity College Institute of Neuroscience, Dublin, Ireland

The hippocampal formation (HF) is known to be critically involved in episodic memory formation, and is also a key site of action of stress. Stress induction in humans results in two main neurometabolic sequelae, the inhibition of RNA/protein synthesis and an increase in

glucocorticoid levels, with both of these changes impacting directly on HF. Prolonged exposure to stress in humans has been shown to lead to hippocampal shrinkage and dysfunction (e.g. in PTSD), while acute stress can result in temporary memory impairment, as in exam-induced forgetting. Research suggests that memory is influenced by stress and the associated rise of glucocorticoids, such as cortisol. Hippocampal-based cognitive functions have been shown to be at particular risk from the deleterious effects of glucocorticoids. However, no large-scale investigation of the effects of stress on hippocampally-based memory has yet been attempted in humans. While human studies have generally found a negative effect of stress and elevated cortisol on memory, animal studies have demonstrated a dose-dependent facilitative effect. These discrepant findings may be a result of methodological limitations in the human literature, which often confound the different stages of memory by elevating cortisol levels prior to encoding, consolidation *and* retrieval. The purpose of the current study was to parse the effects of an acute psychosocial stressor on separate memory processes by varying the timing of the stressor. More specifically, the current study sought to determine the effect of psychosocial stress on distinct memory processes (i.e., encoding and retrieval) using a combination of hippocampally-based behavioral measures (i.e., Visual Paired Associates (VPA) task; Rey Auditory Verbal Learning Test; RAVLT). Psychosocial stress was induced through a series of computerized mental arithmetic challenges as well as a time-limited serial subtraction task in front of an audience, both of which were characterized by uncontrollable and social-evaluative elements. Regarding the pre-encoding stress condition (n=10), participants were first exposed to the 20 minute stress task, followed immediately by encoding of a verbally-based RAVLT word list and a visual-based old/new Visual Paired Associates (VPA) task. Following encoding, participants were submitted to a 20 minute non-stressful attention task (i.e., the 0-back Task). Retrieval was then tested for both the RAVLT word list (i.e., delayed recall) and VPA associates. For the pre-retrieval stress condition (n=10), participants were primarily exposed to the attention task, followed by encoding and stress-induction. Regarding the control condition (n=20), participants were exposed to a similar protocol as pre-encoding and pre-retrieval conditions, however, the control task given was a non-stress eliciting arithmetic task which was both controllable and predictable and completed without a social stress element attached. Salivary cortisol was measured at baseline and 20 minutes after the stressor. It was predicted that pre-encoding stress would lead to greater memory impairment than pre-retrieval stress, wherein the memory trace has theoretically already been consolidated. In response to results emanating from this research, a closer examination of the impact of stress during the reconsolidation phase of memory encoding will be conducted in order to unravel the complex phenomenon whereby “re-activated” memories can be weakened, altered or even erased by inhibited protein synthesis in HF. Investigation of this phenomenon in humans will lead to a clearer understanding of the ways in which learning and memory can be enhanced, as well as the conditions under which it may be impaired or manipulated. On a larger scale, persistent retrieval and reconsolidation of traumatic memories in PTSD and phobia patients is a process that enables such memories to persist. By inhibiting memory retrieval, cortisol may weaken the traumatic memory trace, and thereby reduce symptoms.

INHIBITOR OF HSP70 EXPRESSION QUERCETIN ABOLISHES ANXIOLYTIC EFFECT INDUCED BY THERMAL PRECONDITIONING IN RATS

MV Chernyshev, OA Sapach, Yu F Pastukhov

Institute of Evolutionary Physiology and Biochemistry RAS, St. Petersburg, Russia

Stress protein, or heat shock protein, 70 kDa (HSP70) is one of the most conservative systems of cell and organism protection from various damaging factors (review: Pastukhov, Ekimova, 2005). The most effective method of the enhancement of HSP70 expression in

various organs and tissues, including CNS, is a thermal preconditioning (TP) approach. Protective effects of TP have been elicited in different biological levels, however, so far there is no data related to behavioral studies. The purpose of the present study was to examine an effect of the TP in two standard behavioral tests for anxiety in rats. In order to verify the HSP70 mechanism underlying possible behavioral effects we used an injection of inhibitor of HSP70 expression quercetin. Experiments were carried out in adult male Wistar rats. The TP procedure was conducted inside a thermal chamber in anesthetized animals for 15 min since the moment of the rise of rectal temperature at 41° C. Control group was also anesthetized, but not placed inside the chamber. Quercetin injections were peritoneally performed 4 h before the TP procedure. 24 h later all groups were submitted to the behavioral tests. In the elevated plus maze test, TP group compared to control exhibited a pronounced decrease in the level of anxiety-like behavior scored by percentage of open arm entries (the number and the duration) and the number of unprotected head dips. At the same time, this group exhibited an increase in the levels of exploratory and locomotor activities scored by the number of protected head dips, the number and the duration of open/close arm entries, the number of open arm sectors, the number of transitions between open/close arms, the number and the time of central platform entries. No effect of the TP was elicited in the level of emotionality scored by the number of defecations/urinations, grooming patterns (correct/incorrect), rearing activity and different latencies. In the open field test, TP group compared to control exhibited only an increase in the level of locomotor domain scored by the number of wall ambulations and rearing activity. Treating animals with quercetin abolished or attenuated all the effects induced by the TP. Thus, the results of the study showed that the prior TP procedure may induce an anxiolytic effect against a background of the increased level in exploration and locomotion, with the specifics depending on the behavioral test and indicating the heterogeneity of anxiety manifestation. The inhibition of the behavioral effects by quercetin may prove the implication of HSP70 expression underlying the TP biological protective mechanism.

ROLE OF CHAPERONES 70 KDA IN PATHOGENESIS OF SEIZURES WITH DIFFERENT ETIOLOGY

KA Khudik, LE Nitsinskaya, IV Guzhova, IV Ekimova, Yu F Pastukhov

Institute of Evolutionary Physiology and Biochemistry RAS, Institute of Cytology RAS, St. Petersburg, Russia

One of the most pressing problems of epilepsy research is to find endogenous regulators which possess neuroprotective properties and are able to interfere in the crucial link of convulsive activity generation. It is determined that thermal preconditioning (TP) (method of an increase in concentration of Heat Shock Protein 70 kDa (HSP70)) can possess protective action under stress and in many disorders [review: Pastukhov, Ekimova, 2005]. The aim of the present investigation is to study effects of an increase in concentration of endogenous HSP70 by TP on the severity of seizures and neurological disturbances in rats not predisposed to epilepsy and rats genetically predisposed to epilepsy. To make a comparative analysis we used two models of epilepsy, which are similar to some human epileptic disorders: 1) a model of pentylentetrazole (PTZ, 80 mg/kg) –induced seizures in Wistar rats and 2) a model of hereditary audiogenic epilepsy in Krushinskii-Molodkina (KM) rats. Changes were determined in seizures components in Wistar rats 24 hrs after TP and concentration of inducible Hsp70 (by electrophoresis in polyacrilamide gel and immunoblotting with monoclonal antibody to Hsp70) 6 and 24 hrs after TP. It was found that TP increased Hsp 70 concentration in the following Wistar brain areas: senso-motor cortex, corpus callosum, thalamus, hypothalamus, cerebellum, midbrain, piriform cortex and amygdale; the highest increase was observed in hippocampus 24 hrs after TP. TP resulted in an increase in the latent period (by 2 fold) of

seizures and a decrease in their severity, lethality (by 50%) and the duration of the ataxia symptoms in 48% of rats. Investigation in KM rats revealed an increase in concentration of Hsp70 in hippocampus and piriform cortex 1 day after TP (duration - 7 min) and in midbrain and inferior colliculus 1 and 4 days after TP. TP in KM rats caused a significant increase in the duration of the latent period of seizures induced by sound (intensity 50 dB, frequency 8 kHz) during the 2nd -7th days. The maximum effects were observed on the 4th day (2.7 fold increase) after heat. TP did not reduce the duration of components of audiogenic seizures and neurological disturbances in KM rats. Thus, significant changes in the components of generalized seizures and neurological disturbances induced by PTZ in Wistar rats can be linked to an increase in the level of Hsp70 in many brain areas. An essential delay in seizures in KM rats is associated with the highest increase in the concentration of Hsp70, predominantly in brain areas which are responsible for the initiation of audiogenic seizures. It can be related to abnormalities of the neuromediator brain systems and, possibly, to the production of the regulatory gene dysfunction in KM rats.

EFFECT OF HEAT SHOCK PROTEIN 70 KDA ON BASIC CHARACTERISTICS OF BLOOD DURING THE ENDOGENOUS STRESS IN RATS

KV Lapshina, MN Maslova, IV Ekimova

Sechenov Institute of Evolutionary Physiology and Biochemistry RAS, St. Petersburg, Russia

A rise of the level of bacterial endotoxin lipopolysaccharide (LPS) in blood evokes changes in different physiological systems and biochemical characteristics of living organisms. This kind of stress can be classified as endogenous, and often leads to very serious disorders, such as endotoxaemia and sepsis. Recently we have shown that preliminary injection of heat shock proteins 70 kDa (Hsp70) in rats and pigeons can decrease brain temperature and contractile muscular activity during endotoxaemia [Lapshina, Ekimova, 2007]. Probably, Hsp70 can be considered as the endotoxin antagonist. The goal of our study was to investigate the influence of exogenous Hsp70 on basic characteristics of blood during the endogenous stress caused by LPS in rats. Investigations were carried out in freely moving male Wistar rats. LPS (*Escherichia Coli* 0111:B4 (Sigma) was injected intravenously in a dose of 100 µg/kg. Exogenous Hsp70 (obtained at the Institute of Cytology RAS) was also injected intravenously 15 min before LPS (80 mkg/kg). In the control vehicle, the same volume was injected. Blood samples for the assessment of the number of red and white blood cells, hematocrit and acid resistance of red blood cells were taken an hour after the beginning of the experiment. The number of blood cells was counted using Goryaev chamber, and white blood cells were stained by methylene blue. Acid resistance of erythrocytes was investigated by colorimetric method. It was shown that injections of LPS, Hsp70 and its combined action evoked an increase in the number of red and white blood cells and the hematocrit level. LPS noticeably reduced the acid resistance of erythrocytes, whereas Hsp70 increased resistance in comparison with the control level. After the combined action of Hsp70 and LPS, acid resistance increased in comparison with LPS alone. We supposed that similar changes in blood cells number, hematocrit and fast destruction of erythrocytes may appear due to emotional stress and LPS-induced endogenous stress. The capability of Hsp70 to increase acid resistance can be explained by the release of more resistant young forms of erythrocytes [Maslova et al., 2005, Pastukhov et al., 2005]. In this case, the increase of resistance after the combined action of Hsp70 and LPS may be evidenced by the appearance of young forms of erythrocytes in the blood. The mechanism of this effect is unknown, and requires further experiments.

CALPAIN EXPRESSION IN CNS AND PERIPHERAL CELLS ASSOCIATED WITH EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

MN Karpenko, IN Abdurasulova, VM Klimenko
Institute of Experimental Medicine RAMS, St.Petersburg, Russia.

Multiple sclerosis (MS) is a chronic T cell-mediated autoimmune inflammatory disease which causes neuronal demyelination in the CNS. The corresponding animal model, experimental allergic encephalomyelitis (EAE), is used in evaluating the autoimmune response in CNS and peripheral organ systems. As the calpain family of proteases is implicated in cellular processes such as demyelination, apoptosis and cell migration, we assume that it participates in EAE development. In this study, the calpain expression was evaluated by RT-PCR at the transcriptional level in spleens and spinal cords of animals with EAE. We observed an increase of calpain expression in spleens of rats during the latent period of EAE in comparison with control animals. In spleens of rats with clinical symptoms (paralysis of limbs), calpain expression was decreased to the level of the control group. In spinal cords of rats with severe EAE, calpain expression was significantly increased. We believe that a better understanding these processes could provide avenues for novel therapeutic strategies to treat MS.

BEHAVIOURAL CHARACTERIZATION OF RECOMBINANT ADENO-ASSOCIATED VIRAL (RAAV) VECTOR-MEDIATED HIPPOCAMPAL OVEREXPRESSION OF NEUROPEPTIDE Y (NPY) IN MICE

En-Ju (Deborah) Lin^{1,2}, Herbert Herzog¹ and Matthew During²

¹Neurobiology Program, Garvan Institute of Medical Research, Sydney, Australia;

²Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio, USA.

Neuropeptide Y (NPY) is a 36 amino acid polypeptide widely distributed throughout CNS. It is highly expressed in the hippocampus, and has been shown to be involved in a number of hippocampal dependent functions such as mood regulation, learning and memory. NPY is also implicated in pathological disorders including anxiety-related disorders and epilepsy. In the past decade, there has been significant interest in the modulatory role of NPY in seizures and its potential as a target for novel antiepileptic treatment. We have previously shown that hippocampal NPY overexpression mediated by rAAV in adult rodents significantly protect against kainic acid-induced hippocampal seizures (Richichi, Lin et al., 2004, J Neurosci; Lin et al., 2005, EPN). In order to assess the potential of further development of this gene therapy approach for epilepsies, we evaluated the behavioural effects of adult-onset hippocampal NPY overexpression using a comprehensive behavioural test battery with particular focus on mood and learning/memory. Our results suggest a moderate anxiolytic effect by hippocampal NPY overexpression without significant effect on exploration. No significant effect on cognitive function was observed using the hole board test and passive avoidance test. Interestingly, a moderate but significant increase in immobility time was observed in rAAV-NPY treated mice in the tail suspension test, which suggests an increase in depressive-like behaviour. Taken together, our data suggest that rAAV-mediated hippocampal overexpression of NPY exerts very limited side-effects on hippocampal dependent behaviours and has promising potential in future clinical development as alternative treatment for pharmacoresistant temporal lobe epilepsies.

School Materials

HYBRIDIZING ANIMAL MODELS IN NEUROBEHAVIORAL RESEARCH

JL LaPorte, AV Kalueff

National Institute of Mental Health, NIH, Bethesda, USA

Traditional use of single-domain test batteries for neurophenotyping research is associated with a number of methodological challenges that could be improved using innovative testing techniques. Using “hybrid” protocols that assess multiple domains in parallel may be an effective method of combining experimental paradigms to address these issues by maximizing the number of tested phenotypes per experimental manipulation. Multi-domain models could be created by including a combination of several traditional single-domain models into new research contexts to target additional domains and their interplay. There are numerous benefits to this approach. First, hybrid models require fewer stress exposures than a combination of single-domain models by assessing multiple domains in the same test, saving time and laboratory resources. Using this method will also diminish some undesirable conditioning or stress effects on the animals, which will promote more valid data and limit chances for experimental confounds. Also, when testing distinct domains such as anxiety and depression, this method will allow researchers to model and investigate clinically relevant aspects like comorbidity that single-domain models cannot dissect. The hybridizing approach also enhances the ability to observe a larger spectrum of behavioral phenomena, a fact that aids in the investigation of complex neurophenotypes. This is particularly important when screening pharmaceutical drug compounds or when testing new transgenic or mutant animals. Another very important advantage of using hybrid method is that it can model the “continuum” nature of brain pathogenesis (e.g. transmission from anxiety to depression), which will become increasingly useful considering the new developments in clinical psychiatry. In conclusion, the hybridization strategy offers new developments in behavioral analyses that are lacking in the standard single-domain tests. As such, they enable innovative modeling of neuropsychiatric disorders by more thorough and broader investigation of complex phenotypical characteristics and may represent a solution for today’s neurophenotyping research. This research was supported in part by the Intramural Program of the NIH, NIMH and by a NARSAD YI Award.

REFOCUSING PSYCHIATRIC GENETICS: FROM DISORDERED DOMAINS TO DOMAIN INTERPLAY

AV Kalueff, JL LaPorte

National Institute of Mental Health, NIH, Maryland, USA

The field of biological psychiatry can greatly benefit from new perspectives on the old problems facing researchers. Previously, there have been limited techniques available for the study of emotional states in humans and animals. Behavioral neurophenotyping has been one of the field’s most familiar tools. However, new experimental techniques are needed to ensure further progress in the field. The concept of domain interplay shifts the focus away from singular domains or interactions, and focuses on modeling numerous separate domains and the interface between them. By modeling these phenotypical interfaces, researchers can link domains in relation to each other, thereby modeling an extended conglomeration of interactions that will aid in characterization. In this way, interactions can be modeled across-species and used as a tool for effective translational research, thereby widening the extent of phenotypical characteristics that can be investigated and decreasing the risk of inconsistent

results. The concept of domain interplay encourages research efforts away from the traditional narrow single-domain approaches, and helps alleviate some of the guesswork inherent in endophenotyping and cross-species techniques. The domain-interplay concept promotes correct data interpretation in behavioral paradigms and supports the construct validity of the animal model by harnessing this inherent emotional complexity. Evaluating several domains simultaneously will distinctly improve the ability of the models to mimic the entire pathway of the disorder, instead of a single point along the continuum. Thus, the clinically-relevant aspects of brain disorders, such as comorbidity and disorder pathogenesis, can now be realistically achieved with this concept, opening new paths of research into neurological substrates of pathogenesis. The domain interplay concept provides a design for creating models that exploit the interplay of domains evident in basic and clinical research of many brain maladies. This research was supported in part by the Intramural Program of the NIH, NIMH and by a NARSAD YI Award.

THE USE OF NOLDUS TOOLS FOR STRESS BEHAVIOR RESEARCH

RF Roelofs

Noldus Information Technology, Wageningen, The Netherlands

Introduction: Since the founding of our company in 1987, NoldusIT has evolved into the market leader of behavioral research tools. Currently our software and hardware tools (e.g. The Observer® XT, EthoVision® XT, CatWalk® and Theme®) are used for the collection and analysis of behavioral data in every discipline of behavioral research (e.g. Biology, Psychology, Industrial design, etc). EthoVision® XT is the latest version of our versatile Video Tracking System designed for the collection, analysis and presentation of automatically generated position related parameters (e.g. position, orientation, distance, speed, movement, etc) of freely moving animals. Because EthoVision® XT is a video based system its spatial and temporal resolution is much higher and the data more versatile and reliable than with non video based systems, like photo-beam based systems. Furthermore, its restrictions on the animals' environment is far less pronounced than in any other competitive system, giving the researcher the freedom to gather behavioral data for longer periods in a wide variety of circumstances (e.g. home cage environment, enriched environment). This means it can be used for almost any test available to behavioral researchers that involves movement and position analysis. The video and behavioral data acquired with EthoVision® XT can also be used to analyze behaviors that are not automatically generated (e.g. grooming, rearing, sniffing, head dipping) giving researchers the ability to create the most detailed and refined picture of behavior in almost any situation. During my presentation I will give an overview of how The Observer® XT, EthoVision® XT and Theme® are used by various researchers around the world for gathering, analyzing and presenting stress related behaviors in non-standardized and standardized behavioral tests (e.g. Porsolt (Forced) Swimming Test, Open Field, Elevated Plus Maze, Zero Maze and their variations).

BEHAVIOR RECOGNITION—W5: THE NEXT GENERATION OF TECHNOLOGY FOR BEHAVIORAL RESEARCH

JH Thompson, V Kobla, X Bai, F Li, D Liu, M Sun, Y Liang

Clever Sys Inc., Reston, George Mason University, Fairfax, VA, University of Minnesota, Minneapolis, MN, USA

Introduction: Behavioral researchers today are frequently met with the challenges of finding adequate methods to observe and record animal behavior. Neurological diseases are being characterized by the behavioral phenotypes of mouse models and targeted mutations of

genes expressed in the brain are revealing the underlying mechanisms of behavior. As a result, the most comprehensive maps of the brain include molecular, cellular, system, and behavioral data. All of which are dynamic, interactive, interdependent, and complex processes. Clever Sys. Inc. (CSI), a bioinformatics software company, has devised a system that automatically recognizes and records animal behaviors in a variety of environments depending on individual research needs. Methods: For the purpose of the development of this technology, CSI roughly classifies behaviors into 4 categories: natural, simple, complex, and designed. The patented software is characterized by the ability to capture the entire animal body and discriminate between its individual parts. In addition to approaches in analyzing temporal-space relationships, such as with time sequence-analysis, CSI has developed novel algorithms to determine: what the animal is doing, when the behavior is occurring, where the animal is doing the monitored behavior, and which is which? In specific cases, multiple animals such as mice, drosophila, or zebra fish can be observed for analysis within one apparatus. Results and Discussion: Previous attempts have been made to fulfill this need for automation, such as with the introduction of Photobeam and Video Tracking technologies. Unfortunately, both systems only provide limited information about an animal's location. This is because behaviors that go beyond the measure of location are too sophisticated for either type of technology. CSI has addressed this problem by using novel computer vision and digital video technologies to develop Behavior Recognition technology. Conclusion: Using high throughput video analysis tools allows research to be conducted on a much larger scale at a faster rate; this provides faster results and consequently a more rapid scientific discovery process. This is not to suggest that research be conducted with haste, but rather that CSI has made it is possible to collect data with less restraints, in more natural environments which reflect more unrestrained forms of behavior. This capability will advance the field of behavioral science in ways that have been previously impossible.

THE PAVLOV DEPARTMENT OF PHYSIOLOGY: A SCIENTIFIC HISTORY
VM Klimenko, UP Golikov
Institute of Experimental Medicine RAMS, St. Petersburg, Russia

The organization of the brain and the exploration and understanding of its activity is the boldest challenge for the human intellect, as the brain is the most complicated and highly developed structure created by nature. Throughout history, the challenge of understanding the brain has been taken up by many outstanding people of different nations. However, no final conclusions have been reached, as the questions were too multiple and the means for their exploration were limited. Although the world of science accumulated more and more knowledge about the brain during the 19th century, the activity of higher compartments of the cortex, psychic activity above all, was still out of reach of natural science. Russian physiologist Setchenov was one of the first to lead the way towards solutions in his work *Reflexes of the Brain*. He was followed by Pavlov and his colleagues who studied the same questions in the Department of Physiology at the Imperial Institute for Experimental Medicine in St. Petersburg (IEM).

The Institute of Experimental Medicine. In 1890 in St. Petersburg, the official opening of the Imperial Institute of Experimental Medicine took place. This organization was initiated by Prince Oldenburgsky and was the first Russian scientific research institution in the field of biology and medicine. Pavlov was drawn into the development of the Institute, which included Departments of Chemistry, General Bacteriology, Pathologic Anatomy, Epizootology, and Syphilidology. Pavlov was invited to head the Department of Physiology and guide their researchers - a job he successfully performed for 45 years from 1890 until 1936. In April of 1890, Pavlov accepted the position as Head of the Pharmacology Department at the Military Medical Academy (MMA), which he left in 1895, when he became the Head of the Physiology Department of the MMA. Since the Department did not have sufficient facilities for his studies, students of the

Academy practiced at the Department of Physiology headed by Pavlov at the IEM. At that time, the IEM constructed a new building, specifically equipped for scientific research. Young students evolved into researchers here, under the guidance of Pavlov. Therefore, it can be said that Pavlov's physiological school was actually formed at the Department of Physiology of the IEM. With the aid of the permanent fistulae technique, the Department at the IEM studied the activity of the gastro-intestinal tract, determined the mechanisms of the digestive glands activity, and elucidated the role of the nervous system in regulating these activities. The classic operations of oesophagotomy, isolation of the gastric pouch, pancreatic and bile fistulae and a number of other experimental techniques were developed. Also, the innervations of gastric glands and the physiology of the pancreas were investigated here. In the spring of 1895, Pavlov delivered lectures on digestion at the IEM. During these presentations he reviewed the achievements of the Department in this area of physiology. Those lectures were an important step in bringing order to the vast field of information on nervous regulation of the digestive glands, on the laws ruling the production of digestive juices, and on the interaction of stomach secretion with the functions of the liver, pancreas, and small intestines, as well as with the functions of other parts of the digestive tract. Pavlov demonstrated that the wide use of experimental surgery in long-term experiments on animals gave the opportunity to investigate interactions between the functions and mechanisms of the digestive glands. It was a new group of studies in digestion: exploration of physiological mechanisms of the digestive glands under conditions of long-term experiments on healthy animals with complete and continuously working digestive systems. Pavlov summarized all these achievements in this field in his book *Lectures on the Work of the Chief Digestive Glands* (1897). In this book he added his eighth lecture, "Physiological Data, Human Instinct and Medical Empiricism." A year later the book was published in German after being translated by his pupil Valter. By 1904, when Pavlov was awarded with the Nobel Prize, he had been engaged in scientific and pedagogical activities for 25 years. His studies made a large impact on the scientific community; never had physiology helped clinical medicine so significantly. In his lectures, Pavlov regarded the process of digestion as a physiological conveyer which combined separate organs of the digestive tract into a system. Such an approach helped to create a complete picture of the mechanisms of digestion. At every stage of his research, Pavlov paid great attention to the connection of physiological investigation and clinical medicine. Elaborating on questions regarding the physiology of digestion, he systematically studied the pathology of digestive organs as well. Between 1898 and 1904, studies of the digestive function of the liver and transport of food from the stomach to intestines were carried out under Pavlov's direction. The general coordination of all parts of the digestive tract was revealed as well. Special research was devoted to bile, pancreatic, and gastric juice secretion into the stomach. Conditions for such secretion and its relevance to digestion were determined. Pavlov's research formed the basis for the modern concept of disturbed gastro-intestinal tract function and facilitated the development of adequate therapeutic methods for their treatment. The highest form of appreciation during the period of Pavlov's research was the Nobel Prize which was first given to a Russian scientist in 1904 as a token of acknowledgement of his works on physiology of digestion, which reformed and widened the knowledge in this field. Pavlov's work on the physiology of digestion was a separate and complete set of systemic studies which was internationally acknowledged in 1904. It served as a starting point for a large series of studies that laid the foundation for a new area of physiology—physiology of higher nervous activity, or the theory of conditioned reflexes. The phenomena of "psychic secretion" of digestive glands attracted Pavlov's closest attention. Pavlov endeavored to fill in this "white spot" on the map of knowledge and decided to explore the psychic aspect of the digestive glands activity. This decision was followed by 35 years of tenacious work devoted to the exploration of special brain reflexes which Pavlov termed "conditioned" reflexes. As the history of science shows us, his work on conditioned reflexes brought him more success, popularity and fame than his work on the physiology of digestion, for which he was awarded the Nobel Prize. It is necessary to note that it was Bidder and Schmidt, from Derpt University, who

for the first time in 1852 described gastric secretion from the gastric fistula conditioned by food demonstration. However, at that time the theory of psychic secretion of gastric juice did not attract the serious attention of scientists. The data gained in the Physiology laboratory at the MMA by von Anrep went unnoticed as well, though he was one of the first who, before Pavlov's work, came to understand the role of the nervous system in gastric secretion. Questions were finally answered by Pavlov and Shumova-Simanovskaia and results were published in the article "Gastric Gland Innervation in Dogs." A study carried out by Glinksky, who was a temporary member of the Department of Physiology at the IEM, played a crucial role in the exploration of salivary glands and nervous regulation. It also played a basic role in the physiology of conditioned reflexes. He invented the operation of implanting fistula into the ducts of salivary glands in 1895, and he also performed the first experiments with reflective salivation in dogs. His work was not published because of unknown reasons, but on May 13, 1895, Pavlov presented the results of Glinksky's study at the meeting of the Society of Russian Physicians. Pavlov also wrote in 1902 about the same matter in an article on Dr. Glinksky's technique. From then on, Glinksky's technique was accepted all over the scientific world as the most convenient method of precise and full registration of secreted saliva in long-term experiments. Early in the 20th century, Pavlov's investigations attracted the attention and interest of specialists. In his letter to Prince Oldenburg, curator of the IEM, Pavlov remarked that the Department of Physiology had become a significant attraction for brilliant and loyal scientists not only from Russia, but from other countries. In 1902, Doctors Stensma (Amsterdam), Straub and Fridental (Berlin University), Gross and Professor Konheim (Heidelberg University), and Professor Chermak (University of Halle) worked in the Department under the direction of Pavlov. Pavlov's *Lectures on the Work of the Chief Digestive Glands* (which had been translated into both German and English), along with his lecture delivered at the XIII International Congress of Physicians (where he addressed an international audience for the first time in July, 1900), were the reasons for the growing interest in his work. In his report "Experimental Therapy as a New and Fruitful Technique of Physiological Studies" he did not limit himself to the contents of his latest works. He concentrated on the questions related to "experimental therapy" and announced that physiology, with its special resources and its chances for success, is aimed at such scientific work that completely coincides with the modus operandi of medicine in its treatment of sick human beings. In natural science, one obstacle to understanding the activity of the higher processes of the brain was the initial problem of comprehending the relationship between physical and psychic processes in nature. That is (in a philosophical approach) the question of material and spiritual, or objective and subjective. Throughout the history of science we can find evidence of this debate.

The Birth of a New Era: Conditioned Reflexes. The theory of psychic excitation of salivary glands was elaborated between 1896 and 1901 by Pavlov in co-operation with Wolfson and Snarsky. In Wolfson's dissertation on salivary glands (1899), which was given at the Department of Physiology at the IEM, the psyche was regarded as a special entity regulating the process of salivation. In the dissertation given by Snarsky (1901) on salivary glands functioning, (which was also carried out at the Department of Physiology), the facts were explained from the point of view of zoo-psychologists. Discussing the mechanism of "psychic" salivation, the author compared animals and human beings with their subjective inner world. That approach was not accepted by Pavlov. Snarsky insisted on looking for explanations of the phenomena in the field of the subjective. Pavlov, in his own words, "was astonished by scientific unfruitfulness of such an approach to the problem," and he began to look for another solution. After many hours of speculation and "hard intellectual struggle" he decided to treat psychic excitation as a "pure" physiologist, that is, as an objective external observer and experimenter who deals exclusively with external phenomena and their interactions. This decision was made in 1901. Pavlov was sure that a physiological approach to psychic phenomena exploration would allow the fruitful development of brain physiology, which would explore the role of the brain in organizing interactions between the organism and its environment. The moment in which Pavlov stated his "physiological" approach to the phenomenon of "psychic salivation" is regarded as the birth date

of a new notion called the "conditioned reflex." The first work on conditioned reflexes was carried out, after Pavlov suggested it, by Tolotchinov (1902) who temporarily formed a part of the Department of Physiology. The results were presented in 1902 at the Congress of Physicians and Natural Scientists of the Northern Countries of Europe in Helsingfors (Finland). Tolotchinov described some external conditions under which temporary connections appear in the cortex. He also established the natural reflex generating the natural reflex, its dissipation and restoration, and the possibility of external inhibition of a newly elaborated reflex. The experiments registered not only secretion, but also a motor conditioned reaction. Pavlov, just as many other scientists who were looking for the explanation of the essence of life, was interested in the way in which the brain generated the mind and mentality. All his life he longed to explore the depth of the human psyche. In his first report on the theory of conditioned reflexes, delivered in Madrid at the XIV International Medical Congress in 1903, he said, "Relying on the likeliness and sameness of external phenomena, all objective data obtained in experiments will be used by future science to explain our subjective world. Thus our mysterious nature will be illuminated, and the mechanism of the most interesting human function—of his mind, torments of his mind—will become clear" (p. 119). The varied and in-depth methods of exploring conditioned reflexes chosen by Pavlov in 1901 provided natural science with the opportunity to regain its unconstrained development and enabled it to tread into the "last facet of life"—the mechanisms of the brain's higher activities. The theory of conditioned reflexes developed at the Department of Physiology at the IEM pre-ordained the field of future scientific activities for Pavlov, his practitioners and colleagues. The formation and development of Pavlov's work on the physiology of higher nervous activity were permanently linked with the Physiological Department at the IEM. It was there that Pavlov and his disciples carried out research which permitted Pavlov to give the first lecture on the theory of conditioned reflexes at the Medical Congress of Madrid in April 1903. In his lecture, he demonstrated an objective method of study for higher nervous activity in animals and humans. "Only by the way of objective investigations," Pavlov (1903) stated, "step by step we will reach the complete analysis of that infinite device as a whole, which forms the life on the Earth" (p. 120). The Congress lasted from March 30th until April 26th and there were 6,961 delegates who participated. The Russian delegation consisted of 297 members. Pavlov and his wife were well acquainted with many European capitals, but in Madrid they were deeply impressed by an exposition of Francisco Goya's works in The Prado. They spent a lot of time standing in front of the pictures. Pavlov and his wife also visited Escorial, Toledo and some places in Seville. Beginning in 1904 all efforts of the Department staff were focused on the methodological aspects of exploring the conditioned reflexes. According to Pavlov, high speed accumulation of accurate facts and their easy interpretation presented a drastic contrast with the uncertain and questionable results provided by the subjective approach. It was in 1905 that the method of "artificial" conditioned reflexes was introduced into practice in research. The technique allowed quantitative analysis of the processes of higher nervous activity. As a result, Pavlov formulated the main principle of the conditioned reflex theory according to which the magnitude of the response depends on the intensity of the stimulus. By 1906, almost all types of cortical inhibition had been discovered: conditioned, differentiating, retarding, external and sequential. Basic ideas of the conditioned reflex were formed and conditioned trace reflexes were discovered. Under Pavlov's guidance, investigations were carried out after removing different areas of cerebral cortex (in dogs) to reveal the link between conditioned salivary reflexes and cerebral cortex. Pavlov presented his most important findings in his lecture dedicated to Huxley, which he delivered in London in 1906. At the conclusion of his presentation he felt it necessary to stress his strong belief in the inevitable unity between physiology and medicine. "If the doctor in reality and even more so in ideal is a mechanic repairing a human organism," Pavlov remarked "then any new physiological achievement will sooner or later inevitably expand his power over the mechanism, the power to maintain and repair it" (p. 915). In 1907, Pavlov's disciple Krasnogorsky obtained data on the role of conditioned reflexes in the formation of behavior as he worked with children.

In 1908, Nikiforovskiy took the first steps towards the application of the conditioned reflex technique to pharmacology. Beginning in 1908, investigations in the field of physiology of higher nervous activity under Pavlov's direction were conducted not only at the Physiological Departments at the IEM and MMA, but in the Physiological Laboratory at the Academy of Sciences. However, the Physiology Department remained the main center of scientific work and the experimental base for the development of studies at Pavlov's physiological school. From 1891 to 1917 more than 110 persons worked there under the direction of Pavlov. Dissertations and studies at the Department of Physiology were accomplished by Babkin, Zelioniy, Savich, Orbeli, Krasnogorsky, Zavadsky, Folbort, Tsitovitch, Krestovnikov, Kupalov, Deriabin, Rozhansky and many others. In connection with the development of research on the physiology of higher nervous activity at the IEM, a problem of establishing a special laboratory equipped with soundproof chambers arose. Since the IEM did not have sufficient funds, Pavlov turned to the Ledentsov Fund for monetary assistance. In 1910, at the Society Council Session in Moscow, Pavlov gave a lecture about tasks and arrangement of a model laboratory for studying higher parts of the central nervous system in higher animals. The Society granted Pavlov 50,000 rubles and in 1913 the "Tower of Silence," a three-story building with three soundproof chambers, was built. Five more chambers were added by 1917. In 1911, Pavlov started a broad investigation of cortex inhibition and formulated the major laws of the development of neural processes in the brain cortex. He also defined the notion of two main mechanisms operating in the central nervous system: the mechanism of temporary connection and the mechanism of analyzation. Further on in his scientific investigations, he returned to these definitions repeatedly. Ten years after his first presentation on conditioned reflexes, Pavlov delivered a report called "Investigation of HNA" (higher nervous activity) at the IX International Physiological Congress in Groningen (Holland). Outstanding physiologists such as Sherrington, Starling, Gemmeter, and Fisher participated in the work of the Congress. In his report, Pavlov substantiated his idea that analyzers were a special part of the nervous system. He also presented his research with this perspective based on the idea of a unified center and periphery. In addition, he mentioned the possibility of conditioned reflexes being hereditary, an idea that was later transformed into the question of the genetics of high cerebral functions. During World War I, the two revolutions that followed, and the Civil War, scientific work continued at the DEM. The period between 1918 and 1920 was especially difficult because the country was in ruins, and as a result of starvation and cold it was impossible to experiment on dogs.

Pavlov after the Revolution. Between 1921 and 1923, scientific work in the Department of Physiology at the IEM gradually returned to normal and investigations began again. In Pavlov's report "Normal Activity and General Constitution of Cerebral Cortex", delivered in 1922 at the meeting of the Society of Finnish Physicians in Helsingfors, he distinguished 6 types of events which "embraced the whole HNA without residue". Those 6 events included excitation, inhibition, movement (irradiation and concentration), mutual induction, connecting and disconnecting, and, finally, analysis. It was a report which summarized two decades of the most important results from his work. In 1923 Pavlov published a new book. It was comprised of his articles, reports, lectures and speeches presented in chronological order, so that it reflected the course of development of the theory of conditioned reflexes. Placing special significance on the sixth edition of this book, the last one published during his lifetime, Pavlov wrote in January of 1936 that the book was enriched abundantly—12 new works had been added to it. According to Pavlov, those works clearly demonstrated how immensely the horizon of research had extended. Physiology, psychology (with its practical applications) and pathology (with therapy of the cortex of the brain) had started to join and merge so that they became the same field of scientific work. Judging by the results, this combination has been to their mutual benefit. Between 1925 and 1927, much attention was paid to investigating nervous system types and to studying different kinds of internal inhibition and mutual induction. In 1927 Pavlov published a book on the functions of the hemispheres. In the same year, he suggested that nervous system types be studied on dogs

while researching other questions. The years between 1922 and 1935 were a time of active development of the Physiological Department at the IEM and in-depth study of the physiology and pathology of higher nervous activity under the supervision of Pavlov. In 1923 Pavlov received land to build a special facility for breeding and keeping experimental animals in the vicinity of Koltushi, a village near St. Petersburg. A short time later, Pavlov decided to organize a Biological Station for experimental investigations there. The Station was officially opened in 1926 and it became a base for investigation of conditioned reflexes in dogs in connection with inborn peculiarities of their nervous systems. The stonework laboratory building was completed in 1933. In the same year, the first studies of the higher nervous activity of anthropoids were carried out at the Biological Station under Pavlov's direction. Koltushi became known world wide as the "Capital of Conditioned Reflexes" after the XV International Physiological Congress which took place in Leningrad and Moscow in 1935. Several days before the Congress started, Pavlov initiated the building of a monument dedicated to the dog not far from the Physiological Department building on the premises of the IEM. The statue was created by the sculptor Bepalov. American physiologist and Harvard University Professor Cannon wrote about his meetings with Pavlov in his memoirs: "The last time I saw Pavlov was in Leningrad and Moscow at the conferences of the Physiological Congress in 1935. He was 86 years old then but he looked lively, full of his former energy. I will never forget the day we spent together in the environments of Leningrad, in the huge new buildings of the Institute built by the Soviet Government for Pavlov's experimental works. During our talk Pavlov heaved a sigh and said regretfully that he did not have such huge possibilities 20 years before" (Cannon, 1945, p. 229). In 1918 Pavlov resumed his visits to a mental hospital with the aim of studying the physiological mechanisms of cortical cerebral activities in humans. In those years, Pavlov and his team paid more attention to studies in psychiatric hospitals, which they had started in 890 with the aim of exploring the physiological mechanisms of human cerebral cortex activity. In 1923, Pavlov decided to investigate natural psychopathological syndromes and psychic diseases. In 1931, Pavlov initiated the establishment of two clinics: One based on the neuropsychiatry dispensary and another based on a mental hospital at the Physiology Department. Neurasthenia, hysteria and psychasthenia, narcolepsy, schizophrenia and circular psychosis were investigated in these clinics. The types of higher nervous activity of patients with different neurotic and psychotic sickness dynamics were studied, as well as potential methods of therapy. It should be noted that investigations in the physiology and pathology of higher nervous activity reached their peak in the 1930s. Positive and negative induction phenomena and their temporal and spatial features were discovered with the conditioned reflex method. A concept of sleep, as well as sleeping control methods, was elaborated there. The possibility of producing conditioned reflexes to complexes of irritants working concurrently, or one after another was discovered, as well as producing reflexes to time intervals ("time reflex"). The research on conditioned reflex activity in cases of disturbances in normal higher brain functioning, and understanding the conditions that induce such disturbances, led Pavlov to the elaboration of the concept of four main types of nervous systems. This later formed one of the most important ideas of higher nervous physiological activity. Based on the results of the behavioral investigations on anthropoids, Pavlov proposed the concept of conditioned sensory and signal temporal associations. The latter meant the possibility of forming genetic causative relations between subjects and events in anthropoids. Pavlov stated his belief that it is incorrect to interpret the behavior of highly developed animals relying on the mechanism of conditioned reflex only.

Pavlov and his School. The formation and development of Pavlov's scientific school between 1903 and 1925 was characterized by the dedication of Pavlov and his disciples. For the most part, it was based on questions related to the physiology of higher nervous activity. With Pavlov's guidance and personal participation, the mechanisms of conditioning and the closing function of the brain were studied. The research stated the idea of analyzation and synthesizing activities of the higher brain levels. The role of conditioned stimuli strength was formulated, the main nervous

processes (excitation and inhibition) were characterized, the phenomenon of beyond-limits-inhibition and mutual induction was discovered during this time. Also, the theories of dynamic stereotyping, experimental neuroses, and types of higher nervous activity evolved. The questions of experimental pathology of the higher nervous activity were worked on at the Department of Physiology, and pharmacological substances restoring nervous activity were studied. Results of the work performed at neurological and mental clinics provided a physiological basis for the mechanisms of a number of nervous and mental diseases in humans. Under Pavlov's guidance, Anokhin, Biriukov, Bykov, Ivanov-Smolensky, Mayorov, Orbeli, Razenkov and Speransky worked at the Department of Physiology as well as many other scientists and disciples of the Pavlov physiological school. It was a period when many prominent scientists and representatives of Pavlov's school left the Department of Physiology at the IEM and began to work independently. Among them were scientists who influenced the development of physiology abroad, von Anrep for example . After 1920, von Anrep worked at London and Cambridge Universities, became a Member of Royal Society and for more than 20 years headed the Department of Physiology at the Egyptian University of Cairo. Babkin, who introduced Pavlov's ideas into physiological research in England and Canada, was a Member of Canadian Royal Society. Boldyreff emigrated to Japan in 1918 and in 1922 moved to the USA, where he headed the Pavlov Laboratory at the Sanatorium in the State of Michigan until 1940. In Poland, Konorsky developed neurophysiology, as did Ten-Kate who worked in Holland. Gantt, who worked at Pavlov's laboratories from 1925 to 1929, played a key role in the subsequent development of Pavlov's ideas in the USA. Within Gantt's archives, there is a rich collection of documents connected with Pavlov. Among other things, he organized the Pavlov Scientific Society in the USA. Besides the wide field of investigations in physiology and pathology of higher nervous activity, Pavlov contributed to the development of new trends of research at the Physiology Department. It was because of the physiology and pathology of cortical-visceral relations, first and foremost, that a trend originated at the intersection of physiology of higher nervous activity and physiology of autonomic functions. Investigations in this field began with the work *Development of Urinary Excretion Conditioned Reflexes*, which was carried out in 1926 by Pavlov's disciple Bykov in collaboration with Alekseev-Berkman. By 1931, significant experimental material on cortical regulation of the activities of the internal organs had been accumulated. The second trend of research initiated by Pavlov was the first Russian systematic study of the influence of different health factors in animal and human organisms. In 1931, the further development of these two trends was passed on to the Department of Applied Physiology, which was newly organized at the IEM. In 1931, it was called the Department of Common Physiology and headed by Bykov. It is now called the K.M. Bykov Department of Visceral Systems. In 1933, Orbeli, one of Pavlov's oldest disciples and collaborators, organized the third physiological department within the IEM - the Department of Special and Evolutionary Physiology. The systematic study of some branches of physiology, which had not yet been studied in the Soviet Union, as well as the theories of the evolution of functions of animal and human organisms, were the subject of study in this department. It is interesting to note that Orbeli became successor to Pavlov as the Head of the Physiology Department at the MMA, when Pavlov left in 1925. By the end of Pavlov's life, two physiological departments at the IEM besides the Physiological Department and the Biological Station had been established and were headed by Pavlov's disciples. Both were geared towards the study of physiology and pathology of higher nervous activity. By that time, Pavlov was an Honorary Member of more than 100 Scientific Societies in many countries of the World, including Cambridge University. Pavlov died on February 27, 1936. The last time he visited the Department of Physiology at the IEM, on February 18th of the same year, is commemorated by a calendar on the desk in his office which is now a memorial. The coffin with the body of the Honorary Director of the IEM and Nobel Laureate, was placed for its last farewell in Tavritchesky Palace, the former sitting place of the Duma -Russian Parliament before the Revolution. He was buried in the Academician Yard of the Memorial Cemetery "Litterateurs' Brow." According to the decision made by the Government, his

name was given to the Department of Physiology at the IEM which was founded by him, to the 1st Leningrad Medical Institute (now the St. Petersburg State Medical University named after Pavlov), to the Physiological Institute of the USSR AS (now called the Institute of Physiology named after Pavlov by the Russian Academy of Science), and also to many other research and educational medical institutions. To perpetuate the memory of the organizer and first Head of the Department, Pavlov's office was preserved as a memorial in the Department of Physiology at the IEM, and Pavlov's museum was opened in his apartment on Vassilievsky Island, which is a part of St. Petersburg.

From Pavlov to the XXI Century. After Pavlov's death, the Department at the IEM was headed by Academicians of the USSR AMSci Orbeli (1936 to 1937), then by Kupalov (1937 to 1964), Khananashvili (1965 to 1976) and Vartanian (1978 to 1995). Since 1995 the Department has been headed by Professor Klimenko. Kupalov was the closest disciple and colleague of Pavlov, about whom Pavlov said, "Kupalov is my alter ego." Under the guidance of Kupalov, new regularities were revealed in brain functions. Shortened conditioned reflexes were discovered, the mechanisms of the tonus regulation were found in the cortex, properties of long-term neural processes were studied under normal and pathological conditions and properties of cortical representation of the unconditioned reflexes were characterized. Thanks to the technique of situational conditioning suggested by Kupalov, general regularities of higher nervous activity were studied in animals under conditions of unrestrained behavior and some new causes for experimental neuroses and their mechanisms were revealed. During the same time period another disciple of Pavlov, Abuladze, carried out his investigations. He is the author of original investigations based on the outward extension of the tongue's symmetrical areas. This was used to study the conditions of joint and separate functioning of the brain hemispheres, and of unilateral conditioned reflexes. The experiments carried out under the guidance of Academician Khananashvili resulted in the formation of the concept of integrated systems of conditioned reflexes as the functional units of general behavior. The experiments also helped develop the concept of informational neuroses in animals and humans, as well as ways of their prophylaxis and treatment. Influence of various forms of animal interspecies communication upon the mechanisms of higher nervous activity was studied under normal and pathological conditions. In 1976, Khananashvili left the Department of Physiology to accept the position of Director of the Beritashvily Institute of Physiology in Tbilisi, the capital of Georgia. For two years, the investigations in the Department were continued under the guidance of Professor Silakov. Scientists used microelectrode techniques to reveal the mechanisms of formation of temporal connections. It appeared this was due to the activity of a special group of unspecific neurons called "the learning neurons." Their unique feature involves the ability to establish new functional connections among themselves in the course of conditioning. The concept of microsystems of these neurons as the structural-functional basis had been advanced. Later, when the Department was headed by Vartanian, attention was focused on the question of reinforcement and the role of emotional mechanisms and unconditioned reflex mechanisms in the brain's reinforcement functions. New details were revealed concerning the role environmental agents in neurophysiological and psychophysiological mechanisms of emotional behavior. A number of main structural and functional patterns of the brain's emotional mechanisms were described for animals bred under the conditions of communicative deprivation. Currently, there are three laboratories at the Physiological Department at the IEM: a) The Laboratory of Neurobiology of Integrative Brain Functions, b) The Laboratory of Psychophysiology of Emotions, and c) The Clinical Laboratory of Neurodynamic Correction of Psycho-Neurological Pathology.

Laboratory of Neurobiology of Integrative Brain Functions (Head: Klimenko). The main interests of the laboratory include investigating the conditions through which physiological processes in the brain may transform into pathological conditions. We explore the central mechanisms of nervous and immune system interactions by means of afferent signals involving cytokines and transmission through nervous and humoral pathways from an activated immune system. Cytokines recognized initially as immunopeptides demonstrate distinctive

influence on the brain's functions. They are produced by CNS cells and are ligands of neuronal receptors. Moreover, taking into consideration their participation in the signal transference from the immune system to CNS, and their ability to induce the cascade of regulatory processes, one has every reason to consider that cytokines in the brain act as regulatory peptides. These peptides transfer the signal about immune cell activation to the brain, reorganize the perception and behavior of the individual, and subordinate the current functions to the strategy of survival in the environment. Furthermore, in the central compartment, cytokines play the same role as they do on the periphery, that is—as the mediators of inflammation. Techniques used in exploring the brain's functions cover the subject from the level of conditioned reflexes and integral behavior to the mRNA level of peptides and receptor expressions and of neuropeptide production in the brain. Our experiments include studies of neuromediators and cytokine system interaction in autoimmune neurodegenerative processes in the EAE (experimental autoimmune encephalomyelitis). Completely original data has been obtained regarding the enhancing role of pro-inflammatory cytokine levels in blood circulation and in the brain tissue due to hypoxia, trauma, infection, etc. during critical periods of early postnatal ontogenesis. The increase in cytokine levels correlates with the development of psycho-neurological pathology in these adult animals or during the period of their maturation.

The Laboratory of Psychophysiology of Emotions (Head: Tsykunov). This laboratory's work is directed towards investigating the mechanisms of emotions, emotional disorders and solving the problems of anxiety and depressive conditions. Pavlov was one of the first who introduced the concept of reinforcement into scientific literature. Nowadays, the phenomenon of reinforcement is the central point in different theories of emotions and behavior. Main principles of forming conditioned reflexes—a specially directed control of emotional state—are employed by the staff during investigations of dolphin's purposeful activity in free behavior. Adequacy and agility of emotions are destroyed in affective disorders, especially in depression. While working with our models of depression in rats (as a result of mental trauma, caused by a threat to life, or zoo-social conflicts), it was shown that there were two forms of depressive-like disorders. Those disorders have common depressive symptoms, but they were divided in their structure of modified investigation and aggressive behavior as well as by the level of anxiety. It was shown that those conditions are characterized by opposite shifts in lipid turnover. One result of mental trauma is a decrease in Alfa-cholesterol. Disclosure of the mechanisms of those disorders will enhance our understanding of the pathogenesis of depression and will provide the basis for the development of new drugs.

Clinical Laboratory of Neurodynamic Correction of Psycho-Neurological Pathology (Head: Jakovlev). The role of generating conditioned reflexes in the formation of children's behavior was the subject of exploration by Pavlov's disciple Krasnogorsky. The study was carried out when Pavlov was still alive. On the basis of this research it is possible to conclude that the mechanisms of the feedback a person maintains with the environment play an essential role in developing healthy behavior. Conditions of informational deficit, due to poor environment, often result in brain dysfunctions in children <7-10 years old. A risk factor of alcohol and drug addiction, which has the widest distribution among teenagers, is attention deficit syndrome and hyperactivity disorders (ADHD). Pathogenesis of ADHD, and alcohol/drug addiction is based on the deficit of emotional reinforcement which, in turn, results from an imbalance in the systems of neuromediators, peptides and opioids in mesocortical-limbic structures of the brain. A technique involving outer computerized biofeedback for training children with ADHD was developed at the Department of Physiology. As a result of training with biofeedback, all patients demonstrated positive dynamics of vegetative, emotional, and motor reactions. It has been discovered that the kind of guided parameter (for example: EEG, ECG, breathing, etc.) chosen to work out adaptive self-regulation does not matter. A new stereotype of function regulation is formed in the CNS and new functional nervous connections are established.

Conclusion. Summarizing the scientific history of Pavlov's Physiological Department at the IEM in St. Petersburg, the authors would like to emphasize the importance of Pavlov's

contribution to the world of science. He has created a new system of knowledge and introduced new notions which have become an inherent part of physiology, medicine, psychiatry, psychology and pedagogy.

MORPHOLOGICAL AND CYTOCHEMICAL ALTERATIONS IN THE RAT BRAIN AFTER EXPOSURE TO ACUTE MENTAL TRAUMA

SG Tsikunov, GV Beznin, VI Liudyno, DE Korzhevskii
Institute for Experimental Medicine RAMS, St. Petersburg, Russia

Introduction: Mental traumatic experience in human can result in many psycho-emotional disturbances, indelible structural alterations in the central nervous system (especially in hippocampus) and abnormalities in immune and endocrine system function. In the DSM-IV, this is defined as posttraumatic stress disorder (PTSD). Extreme psychotraumatic influences in rats, as demonstrated in our experiments, lead to sustained non-arresting PTSD-like abnormalities in animal behavior, imbalance of lipid metabolism, dysfunction of neurotransmission and hormonal dysfunction (Tsikunov et al. 2005, 2006). Furthermore, in traumatized animals massive structural lesions were found in tissues of the adrenal glands, the mucous tunic of stomach, and the duodenum. The aim of the present investigation was to detect and characterize morphological and cytochemical alterations in brain of the rats that were exposed to the acute psychotraumatic situation. **Materials and methods:** Here we used a model of PTSD. Experiments were made on Sprague-Dawley rats (200–250 g, 4 months old). Animals were single-exposed to the acute psychic trauma. The rats were exposed to a threatening situation by witnessing the death of a cagemate by a black-tailed python, a predator of the rat. 9 days later, the rats were sacrificed, the brain was taken out and fixed via immersion in the zinc-ethanol-formaldehyde. Preparations were made by the method of paraffin sections. Then preparations were stained by the Nissl's method and also to the revelation of PCNA with and without the background Astra blue stain. **Results:** In animals that were not exposed to the psycho-trauma, very few wrinkled cells were found. In rat brain that was fixed on the 9th day after acute psychotraumatic situation, the following alterations were observed. In the Nissl's stain, wrinkled cells and hyperchromatic cells were detected in many regions of brain at high quantities. A maximum of wrinkled cells were revealed in the hippocampus and basal nuclei. Many wrinkled cells were found in the cerebral cortex. Patterns of cytoarchitecture of hippocampus layers were significantly altered compared with controls. Decreased ordering of neurons and chaotic arrangement of axons and dendrites were also found. A high quantity of wrinkled cells was discovered in the suprachiasmatic nucleus. In staining to PCNA with background Astra blue stain, the intensification of proliferation was revealed in some locations of brain tissue. This phenomenon we considered as a nonspecific glial reparative reaction that accompanied neuronal cell death. **Conclusion:** Thus, the influence of a single psychotraumatic situation can result in structural and cytochemical alterations in various regions of rat brain, especially in hippocampus, basal nuclei and cortex of cerebrum after 9 days. Probable mechanisms of the structural development and cytochemical alterations are hypersecretion of glucocorticoid hormones, lesions of neurons due to excitotoxicity, oxidative stress, a decrease of neuronal progenitor proliferation, disturbances in synthesis of neurotrophic factors, decreased surviving of cells in neurogenesis, and remodeling of dendritic arbor.

CORRECTION OF BEHAVIOR MANIFESTATIONS OF THE POSTTRAUMATIC STRESS DISORDER BY MODULATION OF MONOAMINE SYSTEM ACTIVITY OF THE FEMALE RAT BRAIN

SG Tsikunov, AG Pshenichnaya, AG Kusov

Introduction: Currently, the importance of investigating mental diseases such as depression and posttraumatic stress disorder (PTSD) is increasing. Mental trauma is known to play a considerable role in the pathogenesis of these common disorders. Despite the numerous studies, the neurophysiological mechanisms underlying PTSD remain unclear. Most of the neurobiological investigations on the consequences of psychogenic trauma are performed in male animals. At the same time, there are reports demonstrating sex differences in responses to stress. The data obtained in our laboratory show that both female and male rats develop PTSD after exposure to mental trauma. The disorders in female rats are retained during not less than 2 months and depend on the estrous cycle phase. The noradren-, serotonin-, dopamin-, glutamate-ergic and other stress-activated systems are implicated in depression. Numerous data suggest the influence of estrous cycle, not only on reproductive function, but in non-reproductive behavior. The aim of the present study was to investigate mechanisms of compensation of behavioral and emotional disturbances by analyzing the action of receptor ligands on different neuromediator systems in female rats. **Methods:** A model of mental trauma – the experience of partner death from a predator (python) was used for the formation of PTSD manifestations in rats. A group of female rats (200–250 g) was exposed to the predator for 25 min, and rats who survived this situation were returned to their regular cages. The antidepressants were injected i.p. from 2 to 4 p.m. 1-21 days after the psychotraumatic event. Piribedil (a dopaminergic agonist, 0.72 mg/kg) and fluoxetine (a selective inhibitor of serotonin reuptake, 0.64 mg/kg) were used. 0.9% NaCl was used as a control. The substance was given in doses compared with those used in humans for the treatment of depression. The emotional state of female rats was estimated after psychogenic trauma against the background of the introduction of antidepressants. For assessment of behavioral and emotional state, the following tests were used: open field, elevated plus-maze, intruder-resident and Porsolt tests. **Results:** Injection of piribedil after mental trauma increased the investigative behavior as well as decreased freezing in the open field test. The immobility in the Porsolt test decreased vs. control group. In female rats, a significant inhibition in the elevated plus-maze, and in aggression level in the intruder-resident test was revealed after psychogenic stress following the introduction of piribedil. Fluoxetine in the doses used did not change the behavior of the traumatized rats. **Conclusions:** The results of the present study demonstrate changes in the behavior of female rats that have survived a mental trauma. PTSD-like behaviors were corrected by antidepressants. Administration of piribedil promoted normalization of exploratory activity, and decreased anxiety and aggression. Thus, positive results in correcting emotional disorders in female rats were achieved through activation of dopamine receptors. This suggests the involvement of the dopaminergic system in depression and PTSD.

Our Forthcoming Events

- 12th "Stress and Behavior" Conference - 2nd ISBS congress (May 16-20, 2009, St. Petersburg, Russia)
- 2nd ISBS Summer School on behavioral genetics and neuroscience of stress (May 22-27, 2009, Riga, Latvia)

Conference Secretariat: E-mail: isbs-2008@inbox.ru

Web site: <http://rus-neuroscience-soc.bm-science.com/stress-and-behaviour/>

Presenting Authors Index

Bazovkina, Daria
Chernyshev, Mikhail
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