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"Stress and Behavior"

New Orleans, USA
June 22-25, 2011

IN PARTNERSHIP WITH:

Conference registration: all days 9.00-16.00
Venue: Tulane University Medical School,
1430 Tulane Avenue, New Orleans LA 70112

Day 1. June 22, 2011

Main Auditorium, Tulane University Medical School (1430 Tulane Avenue), Downtown Campus, New Orleans, LA, USA

Opening and Welcoming address

9.00-9.45 Opening Plenary Lecture

THE HOW AND Y OF STRESS, ANXIETY AND OBESITY. Z Zukowska, Department of Integrative Biology and Physiology, University of Minnesota, Minneapolis, MN, USA

Obesity is on the rise but so are levels of stress and anxiety disorders, affecting not only adults but also children. Interestingly, some people lose weight when stressed and anxious, while others ingest calorie-rich "comfort foods". The association of stress hyperactivity, anxiety and obesity and metabolic disorders can be transmitted across generations. While certainly affected by genetics, recent data from epidemiological and animal studies suggest non-genomic, epigenetic effects of environmental stressors. Neuropeptide Y (NPY) is one such stress mediator, abundant in the sympathetic nerves – body's main stress system - and in the brain is a potent hypothalamic stimulator of appetite for sugar-rich foods, implicated in obesity, but also is anxiolytic, improves hippocampal neurogenesis and stress coping. Recent evidence suggests that chronic and/or early developmental stress alters NPY expression in the brain, in part by changing DNA methylation of the NPY gene's rich CpG islands. We found that chronic stress (cold, aggressor) accelerated diet-induced abdominal obesity, atherosclerosis and metabolic syndrome by elevating circulating (platelet) NPY levels and activating its NPY-Y2 receptors (R) in the fat and arteries. Interestingly, the same stress also increased anxiety-like behavior and DNA methylation of the NPY promoter, i.e. presumably silencing peptide expression in the brain. Translating this to humans, higher platelet NPY levels associate with earlier onset and greater severity of coronary atherosclerosis, while reduced (brain-derived?) levels are found in patients with anxiety disorder, PTSD. Recently, we studied if chronic stress can exerts longer term, programming" effects to increase propensity for obesity and anxiety across generations. We hypothesized that such "memory" involves adipose stem cells (ASCs), which, unlike terminally differentiated adipocytes, give rise to new adipocytes throughout the organism's lifetime. To determine if ASCs carry stress memory, we "stressed" murine embryonic stem cells with epinephrine during their adipogenic differentiation. This "stress" dramatically up-regulated NPY mRNA while decreasing DNA methylation of its promoter, and increased adipogenesis, which was prevented by NPY receptor blockade. In vivo, offspring of mice stressed during pregnancy and lactation, initially smaller, grew faster than controls when weaned onto high fat diet, and developed abdominal adiposity, Y2R up-regulation and glucose intolerance, even when they were not themselves stressed. Thus, early developmental stress may epigenetically up-regulate NPY (and/or its receptor genes), specifically in ASCs of the visceral fat, and thus program for future development of abdominal obesity and metabolic syndrome. Whether the opposite happens in the brain, resulting in epigenetically-mediated lower NPY in stress-sensitive areas, thus programming for anxiety remains to be determined.

9.45-10.30 Plenary Lecture

PLASTICITY OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS INDUCED BY EXPOSURE TO CHRONIC STRESS. J Tasker, Department of Cell and Molecular Biology and Neuroscience Program, Tulane University, New Orleans, LA, USA

Stress disorders such as depression are often caused by chronic stress exposure and are characterized by sustained elevated circulating glucocorticoids and hypersensitivity of the neuroendocrine axis to acute stressors. Sustained high glucocorticoid levels suggest a desensitization of the glucocorticoid negative feedback actions and activational hypersensitivity suggests a hyperexcitability of hormone-secreting cells of the hypothalamic-pituitary-adrenal (HPA) axis. We have used a chronic variable stress paradigm as a model for stress disorders combined with whole-cell patch clamp recordings in acute hypothalamic slices to test the hypotheses that chronic stress leads 1) to suppression of the rapid inhibitory actions of glucocorticoids in parvocellular neurons of the hypothalamic paraventricular nucleus (PVN), and 2) to enhanced excitatory synaptic inputs to PVN parvocellular neurons. We showed in previous studies that, in hypothalamic slices from untreated rats, glucocorticoids cause a rapid suppression of glutamate synaptic inputs to PVN neuroendocrine cells by stimulating the synthesis and retrograde release of endocannabinoids. Here we tested whether chronic stress induces plasticity in the rapid glucocorticoid actions and glutamatergic synaptic innervation of PVN parvocellular neurons. We also tested anterior pituitary explants for changes in sensitivity to CRH and the rapid suppressive actions of glucocorticoids on adrenocorticotropic hormone release. We found that PVN parvocellular neurons from chronically stressed rats showed increased glutamatergic synaptic innervation, but did not show any decrement in their sensitivity to the rapid actions of

glucocorticoids compared to control animals. The anterior pituitaries from stressed rats also displayed a facilitated ACTH response to CRH, suggesting hypersensitivity, although the suppressive effect of dexamethasone was not attenuated. Our findings suggest, therefore, that the sensitization to neural activation of the HPA axis following chronic stress is caused by an increase in excitatory synaptic innervation of PVN parvocellular neurons and by the increased sensitivity to CRH of anterior pituitary corticotrophs. Our preliminary findings suggest that the desensitization of the HPA axis to the negative feedback effects of glucocorticoids is not caused by decreased rapid glucocorticoid actions at the level of the PVN or anterior pituitary. This work was supported by NIH MH069879.

12.00-17.00 Conference Symposium 1: GREATER NEW ORLEANS SOCIETY FOR NEUROSCIENCE-SPONSORED SYMPOSIUM: BIOLOGICAL PSYCHIATRY I
Chairs: AV Kalueff, J Tasker (Presentations 30 min)
Room 6065, Tulane University Medical School (1430 Tulane Avenue), Downtown Campus, New Orleans, LA, USA

- G Dohanich (New Orleans, USA) ANXIETY, SPATIAL LEARNING/MEMORY, AND LEARNING STRATEGY
- N Vasudevan, AB Buras, E Landers, N Tien, JP Battle, T Gurley (New Orleans, USA) THYROID HORMONE LEVELS REGULATE ANXIETY IN THE MALE MOUSE
- Y Feng (New Orleans, USA) OVER-EXPRESSION OF (PRO)RENIN RECEPTOR INDUCES BOTH ANGIOTENSIN II-DEPENDENT AND INDEPENDENT OXIDATIVE STRESS IN NEURONAL CELLS
- B Hall (New Orleans, USA) REGULATION OF PROTEIN SYNTHESIS MEDIATES THE RAPID ANTIDEPRESSANT EFFECTS OF NMDA RECEPTOR ANTAGONISTS
- A Zsombok, H Gao, K Miyata, KD Hebert, MD Bhaskaran, AV Derbenev (New Orleans, USA) THE ROLE OF TRPV1 RECEPTOR IN THE HYPOTHALAMUS
- S Drury (New Orleans, USA) GENETIC AND EPIGENETIC INFLUENCES IN EARLY ADVERSITY
- A Kalueff (New Orleans, USA) ZEBRAFISH MODELS OF BRAIN DISORDERS

ANXIETY, SPATIAL LEARNING/MEMORY, AND LEARNING STRATEGY: THE ROLES OF TRAIT ANXIETY, BIOLOGICAL SEX, AND MUSCARINIC RECEPTORS IN THE USE OF COGNITIVE STRATEGY IN PREPUBERTAL RATS. G Dohanich, E Grissom, W Hawley, Department of Psychology, Tulane University, New Orleans, LA, USA

When navigating spatial mazes, rats typically employ learning strategies that depend on mediation by the striatum (stimulus-response strategy) or the hippocampus (place strategy) with both areas influenced by the output of the amygdala. Various factors moderate the type of strategy used to learn tasks that can be solved by either strategy, including individual differences in anxiety levels. Our primary hypothesis is to determine if trait anxiety in young male and female rats influences the choice of learning strategy used to solve a maze task. A secondary hypothesis is to determine if a relationship between anxiety and strategy is correlated with cholinergic muscarinic receptor binding in the striatum, hippocampus, and amygdala. Methods: At 26 days of age, anxiety levels of male and female Long-Evans rats were assessed during a five-minute test on an open field. The following day, learning strategy was determined using a modified version of a visible platform water maze task that could be solved by employing either a stimulus-response strategy or a place strategy. After 8 training trials during which rats learned to escape to a stationary visible platform, a probe trial was conducted in which the visible platform was relocated to the opposite quadrant of the circular pool. Rats that swam directly to the platform in its new location were identified as stimulus-response learners and rats that returned to the previous location of the platform were identified as place learners. Following behavioral testing, in vitro receptor autoradiography with tritiated quinuclidinyl benzilate was employed to measure muscarinic receptor binding in the striatum, hippocampus, and amygdala. Results and Discussion: Prepubertal male rats preferred a stimulus-response strategy over a place strategy, in contrast to age-matched females that displayed no strategy preference. Furthermore, the male preference for a stimulus-response strategy was strongly linked to higher levels of trait anxiety. Additionally, cholinergic muscarinic receptor binding was elevated in the striatum and the amygdala of rats using a stimulus-response strategy compared to rats using a place strategy. Our results indicate that the use of a stimulus-response strategy was linked to higher levels of anxiety in prepubertal male rats, but not female rats. This behavioral profile was associated with higher muscarinic binding in the striatum and amygdala, areas that mediate stimulus-response strategy and anxiety. Research Support: Phase II Research Enhancement Award, the Weiss Presidential Fellowship Award, Louisiana Board of Regents PFUND Award.

THYROID HORMONE LEVELS REGULATE ANXIETY IN THE MALE MOUSE. N Vasudevan, AB Buras, E Landers, N Tien, JP Battle, T Gurley. Neuroscience Program, Department of Cell and Molecular Biology, Tulane University, New Orleans, LA, USA

Thyroid hormones (T3 and T4) and their receptors (TR) are required for mammalian central nervous system (CNS) development, with hypothyroidism linked to mood disorders in man. Previous behavioral studies have shown that mutations that lower TRα1 receptor affinity to thyroid hormones lead to anxiety phenotypes and hippocampal dependent memory impairment as well as locomotor dysfunction (cerebellar dependent) in mice. The aim of our current set of experiments is to establish a mouse model of moderate anxiety, using either ligand loss or ligand supplementation, with subsequent analysis of differences in CNS function and neuromorphology. Increased anxiety has been linked to differences in GABAergic neurotransmission and possibly synaptic plasticity, though the mechanisms are largely unknown. Methods: Male C57BL/6 mice were rendered hypothyroid or hyperthyroid by the addition of methimazole and potassium perchlorate in the drinking water. At the end of six weeks of treatment, hypo and hyperthyroid mice were compared to euthyroid controls in behavioral tests that determine anxiety phenotype, aversive stimulus memory and locomotor activity. After behavioral testing, Golgi impregnation was used to compare the dendritic spine densities in the limbic system (hippocampus and amygdala) of these animals. In addition, GABAergic neuron numbers in the limbic system are evaluated for all treatment groups, using immunohistochemistry. Results and Discussion: Behavioral analysis indicates that the hypothyroid mice show the most anxious phenotype in the elevated plus maze and light dark transition tests, while the T3 and T4 supplemented hyperthyroid mice were able to overcome these phenotypic abnormalities. The open field results demonstrate that these anxiety phenotypes are not a result of altered locomotor activity. Results from dendritic spine density studies and immunohistochemistry in the limbic system will also be presented. Research Support: A Buras was supported by the Summer Neuroscience Program (2010), NV is supported by Tulane Startup Funds.

OVER-EXPRESSION OF (PRO)RENIN RECEPTOR INDUCES BOTH ANGIOTENSIN II-DEPENDENT AND INDEPENDENT OXIDATIVE STRESS IN NEURONAL CELLS. Y Feng, Department of Physiology, Tulane University School of Medicine, Tulane Hypertension and Renal Center of Excellence. New Orleans, LA, USA

Oxidative stress plays a pivotal role in the central regulation of BP. Recently, a new component of the brain RAS was discovered and named the (pro)renin receptor (PRR). The binding of renin or prorenin to PRR promotes angiotensin-II (Ang-II) generation, and activates both Ang-II dependent and independent signaling pathways. Ang II binding to angiotensin type 1 (AT1) receptor activates important signaling cascades and stimulates reactive oxygen species (ROS) generation, thus leading to hypertension. However, there is limited information on the role of PRR in oxidative stress in neurons. Methods: Neuro-2A cells were infected with adeno-associated virus coding human PRR (AAV-PRR) or control virus (AAV-GFP) in a concentration of 10-5v.g./cell. Cells were incubated with human prorenin (2 nM) or saline, with or without AT1 receptor blocker, losartan (1 mM) 72 hours after virus infection. ROS generation was evaluated by dihydroethidium (DHE, 5 mM) staining. Using superoxide dependent lucigenin chemiluminescence, NADPH oxidase activity assay was measured to determine the contribution of ROS generation derived from NADPH oxidase. Results and Discussion: The ROS levels (Relative Fluorescence Units) and NADPH oxidase activity (Relative Luminescence Units) was significantly increased in cells infected with AAV-PRR (2.2 ± 0.2; 1.4 ± 0.1) compare to control virus (1.0 ± 0.1; 1.0 ± 0.1; $P<0.05$) respectively. Human prorenin further increased the DHE staining and NADPH oxidase activity (4.0 ± 0.3; 1.6 ± 0.1) compared to saline in cells over-expressing PRR ($P<0.05$). Interestingly, losartan did not block the ROS generation and NADPH oxidase activity induced by AAV-PRR (2.3 ± 0.1; 1.3 ± 0.1), while it inhibited both ROS generation and NADPH oxidase activity (2.4 ± 0.2; 1.1 ± 0.1) induced by the addition of prorenin into AAV-PRR infected cells ($P<0.05$). The data suggests that over-expression of PRR alone in neuronal cells induces Ang II-independent activation of NADPH oxidase and oxidative stress. However, in the presence of prorenin, PRR over-expression activates Ang II-mediated activation of NADPH oxidase and ROS generation in neuronal cells indicating a possible new pathway for oxidative stress in the central nervous system. Research Support: NIH1P30HL101285.

REGULATION OF PROTEIN SYNTHESIS MEDIATES THE RAPID ANTIDEPRESSANT EFFECTS OF NMDA RECEPTOR ANTAGONISTS. B Hall, Tulane University Department of Cell and Molecular Biology and the Neuroscience Program, New Orleans, LA, USA

Chronic stress can lead to increases in depressive behavior. Interestingly, in treatment-resistant depressed patients n-methyl d-aspartate receptor (NMDAR) antagonists promote rapid antidepressant effects. Data suggest that this may be due to NMDAR-dependent regulation of the mTOR protein translation pathway, leading to an increase in synapse number in the prefrontal cortex. However the cellular mechanisms

by which this occurs are unclear. We are investigating the mechanisms by which NMDARs regulate protein synthesis in cortical neurons. NMDARs are heteromeric tetramers and in the cortex each receptor contains two GluN1 subunits and a complement of either GluN2B or GluN2A subunits. We have recently shown that GluN2B-containing NMDARs are uniquely linked to a cellular signaling pathway that regulates protein translation in cortical neurons. We observed a strong increase in protein translation following in vivo or in vitro suppression of GluN2B function. Importantly, our data show that suppression of protein translation by GluN2B-containing NMDARs is not rescued by replacement with GluN2A. Our data also suggest that the observed specificity of GluN2B function is via its unique interaction with the protein effector calcium calmodulin kinase II (CaMKII). GluN2B-mediated signaling specifically suppresses synaptic proteins including AMPAR subunits thereby limiting synaptic current at cortical synapses. Taken together, these data predict that GluN2B-mediated signaling suppresses protein synthesis in cortical neurons and antagonism of this pathway leads to the rapid antidepressant action of NMDAR antagonists. Research Support: This work is being supported by NSF (CAREER award #065374) and the Tulane School of Science and Engineering.

THE ROLE OF TRPV1 RECEPTOR IN THE HYPOTHALAMUS. A Zsombok, H Gao, K Miyata, KD Hebert, MD Bhaskaran, AV Derbenev. Departments of Physiology and Medicine, School of Medicine and Neuroscience Program, Tulane University, New Orleans, LA, USA

Neuronal regulation of glucose homeostasis depends on the activity of the autonomic nervous system controlled by hormones and nutrients. Preautonomic neurons in the paraventricular nucleus (PVN) of the hypothalamus regulate sympathetic and parasympathetic output to the liver participating in the regulation of hepatic glucose production. Patients with diabetes mellitus have altered level of endogenous cannabinoids (eCB) and some eCB ligands such as anandamide (AEA) or NADA, recently called endovanilloids, activate both cannabinoid type 1 (CB1) and transient receptor potential vanilloid type 1 (TRPV1) receptors in the brain. The involvement of peripheral TRPV1 receptor in the regulation of energy and fat metabolism was discovered. However, the role of central TRPV1 on glucose metabolism is unclear. Methods: Patch-clamp recordings from control and type 1 diabetic (T1D) mice were performed to characterize synaptic properties of liver-related PVN neurons identified with PRV-152 during control and diabetic conditions. Immunohistochemistry was used to demonstrate localization and co-localization of TRPV1 receptor and insulin receptor substrate 2 (IRS2) in liver-related preautonomic neurons. We revealed TRPV1 receptor protein expression levels in control and T1D mice by using Western blot. Immunohistochemical studies identified abundant TRPV1 receptor and IRS2 expression in the PVN and co-localization of TRPV1/IRS2 on liver-related preautonomic neurons. Administration of capsaicin, a TRPV1 receptor agonist increased mEPSCs frequency in liver-related PVN neurons in control but not in T1D mice. Pre-application of insulin restored TRPV1 receptor activity in a PKC/PI3K-dependent manner. There was no difference in total TRPV1 receptor protein expression, however increased phosphorylation of TRPV1 receptor was observed in T1D mice, suggesting acute desensitization of TRPV1 receptor. Therefore our data suggest that insulin deficiency and/or hyperglycemia affect the activity of TRPV1 receptors; which can contribute to autonomic dysfunction and dysregulation of neuronal regulation of glucose metabolism. Our data support the hypothesis that central autonomic circuitry is altered in type 1 diabetes. Research Support: Supported by AHA 10GRNT4540000, NIH 2K12HD043451 for AZ, NHLBI R21HL091293, R21HL091293-01A1S1 for AVD.

GENETIC AND EPIGENETIC INFLUENCES IN EARLY ADVERSITY. S Drury, Tulane University Medical School, New Orleans, LA, USA

Across studies early adversity is a risk for a range of negative outcomes. While clear evidence for genetic influence and gene x environment interactions exist the variation in findings indicate more complex models are needed. Recent theories have begun to challenge the vulnerability/resilience concept of gene-environment interactions suggesting instead that these interactions are better characterized in terms of biological sensitivity to context. In this model genetic variation results in greater sensitivity to the environment, for better or worse. Further the influence of epigenetic changes may be additional potential contributors. Early care in institutions (orphanages) is an extreme form of early adversity and a known risk factor for a range of psychological, cognitive, and physical negative outcomes. Children with a history of institutional care who are subsequently placed in an enriched caregiving environment have some recovery in each of these domains, however this recovery is not uniform. The Bucharest Early Intervention Project (BEIP) is the only randomized controlled trial of foster care compared to continued institutional care. Within this unique study we tested a model of genetic plasticity with indiscriminant behavior, a consistent psychological and behavioral construct consistently associated with institutionalization and also examined the association between cumulative exposure to institutional care and telomere length, one epigenetic marker previously linked to psychosocial stress and early adversity. The Bucharest Early Intervention Project (BEIP) is the first randomized controlled

trial of foster care placement as compared to continued institutionalization. 136 children, after a comprehensive baseline assessment, were randomized to either continued institutional care (CAU), n=68, or foster care placement (FCG), n=68, at less than 31 months of age and followed longitudinally. Indiscriminant behavior was assessed at four time points by caregiver report using the same validated semi-structured interview, the Disturbances of Attachment Interview (DAI). We examined the association of indiscriminant behavior at each time point with functional polymorphisms in two genes previously associated with differential susceptibility to early adversity, Brain Derived Neurotrophic Factor (BDNF) val 66met and the serotonin transporter (5HTT) 5httlpr. We additionally explored the hypothesis that the met allele of BDNF and the short allele of the 5httlpr would reflect elevated sensitivity to change in the caregiving environment such that they would be associated with the highest level of symptoms in the CAUG but the lowest level of symptoms when children were placed in foster care. Finally as these two polymorphisms have previously been found to have an additive influence on outcomes we explored the association between indiscriminant symptoms and a combined plasticity genotype (met allele carriers and s/s homozygotes). To examine the association between cumulative exposure to institutional care and telomere length we utilized quantitative PCR to determine the Telomere repeat unit to single copy (T/S) ratio when children were between 6 and 10 years of age. We examined the association between telomere length and cumulative % of time in institutional care prior to 54 months of age. Results: No genotype was associated with gender, ethnicity or group (NIG, CAU or FCG). Genotypes were all in Hardy-Weinberg equilibrium and allele frequencies were similar to those reported in other studies. T/S ratio was not associated with gender or ethnicity. An interaction between group status (FCG or CAUG) and genotype was detected at multiple time points. Children with the s/s 5httlpr genotype or carriers of the met 66 allele in BDNF demonstrated both the greatest decrease in DAI scores in the FCG and the highest persistent rate of indiscriminate social behavior in the CAUG. Regression analysis revealed that 5httlpr genotype had the greatest impact on change in DAI score between baseline and 42 months (p=.02, r2=.17) while the greatest impact of BDNF genotype occurred between 30 and 54 months (p=003; r2=.11). Children with both s/s 5htt genotype and met/ allele carriers of BDNF in the CAUG had the highest number of indiscriminate symptoms at 54 months while those with the same genotype in the FCG had the lowest number of symptoms at 54 months. A significant negative correlation between T/S ratio and percentage of time was observed. Children with greater exposure to institutional care had significantly shorter relative telomere length in middle childhood. Gender modified this main effect. The percentage of time in institutional care at baseline significantly predicted telomere length in females, whereas the percentage of institutional care at 54 months was strongly predictive of telomere length in males. Discussion: These findings represent the first known genetic associations with indiscriminant social behavior. This is the first study, to our knowledge, to demonstrate genetic biological sensitivity to context in the same children exposed to well-defined changes in the caregiving environment associated with institutional rearing. This is also the first study to demonstrate an association between telomere length and institutionalization, the first study to find an association between adversity and telomere length in children, and contributes to the growing literature linking telomere length and early adversity. These findings add to the growing literature demonstrating biological sensitivity to context. Further these findings demonstrate that early adversity has influences down to the cellular level and highlight the need for early intervention for at risk children in adverse environments. Research Support: NARSAD, Tulane University CTREC, Center on the Developing Child at Harvard University, Catherine T. MacArthur Foundation, Binder Family Foundation, NIH.

ZEBRAFISH MODELS OF BRAIN DISORDERS. A Kalueff, Tulane University Medical School, New Orleans, LA, USA

Our lab utilizes adult zebrafish to better understand the behavioral and neuroendocrine consequences of several common drugs of abuse. These psychoactive substances can be grouped into four broad categories: stimulants, depressants, narcotics, and hallucinogens. While members of each group share unique behavioral and physiological effects, and often, conserved pharmacological profiles underlying these effects, all drugs of abuse have at least one common characteristic; they have the potential to make patients “feel good”. That is to say, drug action results in bodily effects with some reinforcing property. Examples may include behavioral inhibition and the anxiolytic nature of central nervous system depressants, such as ethanol, the barbiturate pentobarbital, and the benzodiazepines diazepam (Valium) and chlordiazepoxide (Librium) used in our lab. Morphine and the herbal supplement kratom, examples of opioid narcotics to which we expose zebrafish, are often abused for their analgesic effects, while stimulant abuse, including the widely consumed drugs caffeine and nicotine, is reinforced through increased energy and attention. Lastly, by exposing zebrafish to lysergic acid diethylamide (LSD) and 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), we are able to investigate the reinforcing alterations in perception, emotion and cognition brought on by psychedelic hallucinogen abuse. By studying zebrafish behavior in multiple paradigms that capitalize on

innate behaviors of these fish to novelty, predators and other aversive stimuli, as well as zebrafish social interaction and shoaling behavior, we are able to gain insight into the effects elicited by exposure to each of these drugs of abuse. Moreover, paralleling behavioral results with physiological endpoints, such as cortisol levels (a biomarker for stress), further validates our conclusions. In this way, research in our lab strives to contribute to a more comprehensive understanding of the mechanisms of action and behavioral consequences of drugs of abuse in zebrafish. Ultimately, these conclusions may be translated to humans, expediting the development of more effective ways to treat and prevent drug abuse. Research Support: NIH/NIDA, LA BoR P-Fund grant, Tulane Synergy Pilot grant.

16.20-17.00 Conference Presentation:
DENDRITIC ALTERATIONS AND NEUROCIRCUITRY ABNORMALITIES IN STRESS- AND NEUROPSYCHIATRIC-RELATED DISORDERS. RF Mervis, Department of Neurosurgery, Center of Excellence for Aging and Brain Repair University of South Florida College of Medicine, Neurostructural Research Labs, Inc., Tampa, FL, USA

The dendritic arbor of the typical neuron of the mammalian brain comprises over 95% of the volume of the neuron. In addition, the dendrites of almost all neurons are embellished with thousands of dendritic spines which serve as the post-synaptic loci for the transfer of information between neurons. Morphometric assessment of dendritic branching and spines of a neuronal population therefore represents a critical index of the microcircuitry of those neurons. Dysmorphic alterations in these dendritic parameters include both atrophic and neuroplastic changes which can influence transfer of information -- disruption of which ultimately may be reflected by changes in learning, memory, or behavior. Here, I will present examples of these scenarios which will demonstrate how neuroplastic and/or neurodegenerative changes in dendritic branching and spines may impact on brain circuitry and behavior.

Day 2. June 23, 2011

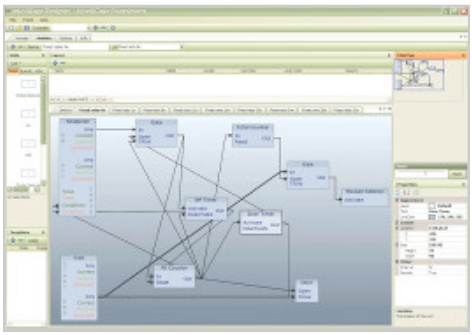
Main Auditorium, Tulane University Medical School (1430 Tulane Avenue), Downtown Campus, New Orleans, LA, USA

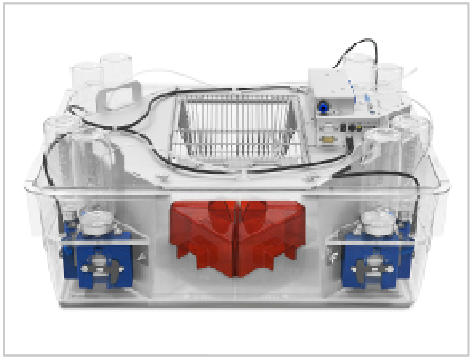
9.00-9.45 Special Plenary Lecture:
DEPRESSION: ARE CYTOKINES THE MEDIATORS? AJ Dunn, University of Hawaii, Hawaii, USA

Stress is associated with a rapid activation of noradrenergic systems both in the brain as well as the peripheral sympathetic nervous system. In the central nervous system, the noradrenergic activation is thought to be arousing, and to focus attention on novel stimuli in many sensory modalities (sight, hearing, smell, etc.). Brain serotonergic systems are also activated, and free tryptophan is increased. The adrenal medulla secretes epinephrine and norepinephrine into the circulation and the sympathetic nervous system adds systemic local norepinephrine increasing blood flow to vital organs and mobilizing glucose stores. The adrenal cortex reacts to corticotropin (ACTH) secreted by the pituitary synthesizing and secreting corticosteroids (cortisol in most species but corticosterone in rodents) which contribute to the mobilization of energy stores, and also affect the expression of some brain genes. Because infectious diseases are considered stressful, we studied the effects of infecting mice with influenza virus, and discovered that the infection was associated with activation of the HPA axis, as well as a substantial activation of noradrenergic and serotonergic neurons in the brain, along with increases of free tryptophan. This coincided with the publication by Besedovsky that minuscule doses of the cytokine interleukin-1 (IL-1) administered peripherally activated the HPA axis, increasing circulating concentrations of ACTH and corticosterone. Thus we tested the effects of peripheral administration of IL-1 to mice, and observed that in addition to the HPA axis activation, brain noradrenergic and serotonergic systems were activated, and brain concentrations of tryptophan were increased, mimicking the effects of physical stressors. These neurochemical and endocrine responses to IL-1 administration were accompanied by behavioral changes resembling those of sick animals (sickness behavior). Reduced activity and food intake and decreased interest in exploration, and in sexual activity, especially in females. The behavioral responses also resembled those observed in depressed patients, and induced responses in animals in the standard tests of depression in rodents, such as the forced swim and tail-suspension tests. This finding led to the suggestion that IL-1 may be the mediator of depressive illness. A major problem with this IL-1 hypothesis of depression is that numerous studies have failed to demonstrate any increase in circulating concentrations of IL-1 in depressed humans, supported by several meta-analyses. Many publications incorrectly cite the studies of Maes in which he studied the secretion of IL-1 in response to

endotoxin (LPS) by white cells derived from depressed patients. Proponents of the “IL-1 mediates depression” hypothesis, retreated to a “cytokines” cause depression hypothesis. Numerous studies have measured circulating concentrations of cytokines in depressed individuals, and very few have shown any elevation of plasma concentrations of IL-1 and most of the meta-analyses are negative. Interestingly, several studies of depressed individuals have indicated elevations of interleukin-6 (IL-6), which has not been shown to induce depression-like symptoms in animals or humans. To save the IL-1 hypothesis, some have retreated to an “IL-1 in the brain causes depression” hypothesis. This is supported by only one study in a very small number of patients (13) indicating increases of IL-1 in CSF (Levine et al., 1999). Research Support: National Institutes of Health (NIMH & NINCDS).

9.45-10.30 Conference Presentation:
INVESTIGATION OF RODENT BEHAVIOR IN THE HOME CAGE INCREASES ANIMAL WELFARE. H. Russig, TSE Systems GmbH, Bad Homburg, Germany





Investigations of genetically modified laboratory rodents provide valuable insight into underlying mechanisms of various human diseases and can provide new tools for drug development. A major step during animal model development is the intensive in-vivo phenotyping and behavioral characterization of animals. Classical behavioral phenotyping requires large sets of animals investigated in batteries of different behavioral tests resulting in substantial experimenter-induced handling stress and data variability. In order to increase throughput and animal welfare, TSE Systems within recent years developed automated home cage test technologies such as PhenoMaster or IntelliCage. The PhenoMaster represents a modular automated test system to investigate behavioral and metabolic alterations in mice or rats 24 hours per day. Similarly, the IntelliCage is a unique solution for automated monitoring of behavior under stress-free conditions within social groups and allows the application of a variety of freely programmable mainly cognitive tasks, usually tested in classical behavioral test batteries. Behavioral domains covered by IntelliCage range from spontaneous behavior such as exploration or anxiety to complex behavior such as discrimination learning or spatial memory. Both systems exclude animal handling induced stress, ensure high animal welfare, and require a reduced number of animals for comprehensive phenotyping. The use of automated systems increases throughput assures high standardization of test procedures and comparability of data! In conclusion, automated home cage test systems open new dimensions for a variety of low-stress in-vivo research approaches in biomedical and preclinical science. A striking example of this home cage monitoring strategy the IntelliCage will be presented. IntelliCage is a novel approach for studying the cognitive behavior of small lab rodents, without handling by the experimenter. The system takes advantage of transponder technology for individual recognition and shaping of animal behavior. IntelliCage records and tests a wide range of behavioral abilities: exploration, approach/avoidance, spatial preference, spatial patrolling (including short-term spatial memory tasks), spatial avoidance and tempo-spatial learning. Individual mice are easily trained to work in the conditioning corners and can be subjected to long lasting operant learning schemes. BENEFITS AT A GLANCE: Opportunity to study learning behavior of mice (and other rodents) without stress and in a social context; Each IntelliCage unit allows simultaneous monitoring of up to 16 mice; Intellectually enriched” environment can be provided, the degree of the challenge being variable; Standardization of procedures.

10.30-11.15 Conference Presentation:
NOLDUS INFORMATION TECHNOLOGY: TOOLS AND TECHNIQUES FOR RESEARCH ON STRESS BEHAVIOR. A Macbeth, Noldus Information Technology, Leesburg, VA, USA

Since our founding in 1989, Noldus IT has evolved into the worldwide market leader for behavioral research tools. We supply researchers with a variety of software and hardware tools that are widely used for

the collection and analysis of behavioral data in every discipline of behavioral research (e.g. biology, psychology, usability, industrial design, etc). Specific to research into stress-related behaviors we offer EthoVision® XT and The Observer® XT, which can be used for gathering, analyzing and presenting data from relevant animal tasks (e.g. forced swim test, open field, elevated plus maze, and elevated zero maze) as well as data from human participants. 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. EthoVision® XT offers a number of advantages over other systems, including: 1) use of video for tracking, resulting in higher spatial and temporal resolution and more reliable data than with non- video based systems (e.g. photo-beam based systems). 2) Tracking can occur in any environment, 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, maze, open field, tanks, well plates, etc). 3) We offer modules to provide the researcher full control over tracking. In addition to our established Multiple Arena Module (track animals in several arenas simultaneously) and Multiple Body Point Module (track head, center and tail-base points), we have added the Social Interaction Module (track multiple subjects together to obtain social parameters) and the Trial and Hardware Control Module (interface with external hardware devices, allowing them to be used during live tracking of subjects; behavioral events displayed by the subject can even be used to control the external devices). 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. However, if greater coding detail is desired, particularly with human participants, then The Observer®XT is the more appropriate software. With The Observer®XT the user can set up a customized coding scheme to accurately describe behaviors in a quantitative fashion. In our newest version, we fully support integration of physiological data with behavioral analysis, allowing for greater insight into your participants' responses. Combined, these two products highlight the newest technologies in the industry available for studying stress-related behavioral phenotypes in both animal and human models.

11.30-12.00 Conference Lecture:

JUVENILE ABUSE INDUCES A SEX-SPECIFIC PATTERN OF ANXIETY AND DEPRESSION-LIKE BEHAVIORS IN ADULT RATS. B Cooke, J Weathington, A Arnold, Neuroscience Institute, Georgia State University, Atlanta, GA, USA

The rate of depression and anxiety is approximately two times higher in women than in men, a sex difference that emerges during puberty. The origin of this sex difference in mood disorders is unknown, although it has been suggested that this may be due to an increased susceptibility of females to stress. Adverse early experience is a major risk factor for anxiety and depression, and it encompasses a broad range of experiences including physical and sexual abuse, and bullying. To determine whether juvenile abuse (JA) induces mood-disorder like behaviors in rats, we subjected juvenile male and female rats to repeated attacks from aggressive adult males and tested them for depression- and anxiety-like behaviors several weeks later. Methods: Beginning at P28, each juvenile was placed into the cage of an aggressive male. It received one 10-minute JA episode with a different aggressive resident each day. Males and females received equal numbers of kicks, dominance postures, and pins over the course of 10 episodes. Controls were treated identically, except that they were placed into a clean empty cage. As adults, the rats were tested in the Porsolt forced swim, the elevated plus maze, a social interaction test, and the open field test, all in counterbalanced order. After the first forced swim, blood was sampled to measure stress-evoked corticosterone, and at the circadian nadir, blood was collected for baseline levels of corticosterone. Results and Discussion: Juvenile abuse induced mood disorder-like behaviors in a sex-specific manner. Abused females were more prone to learned helplessness in the forced swim test compared to their controls and males; JA increased anxiety in the elevated plus maze in both sexes, but the effect of JA was greater in females; abused females made fewer investigatory approaches during the social interaction test, whereas JA had no effect on males. Juvenile abuse also sensitized the hypothalamic-pituitary-adrenal axis in a sex specific manner: JA females had significantly higher swim stress-evoked corticosterone levels than their controls, whereas JA had no effect on stress-evoked corticosterone levels in males. In contrast, baseline levels of corticosterone were unaffected by JA in males and females. Because the level of JA was equivalent across the sexes, these results indicate that female rats are more susceptible to JA than males, a pattern that resembles clinical findings in humans. JA may thus potentially be used as a model of sex differences in the susceptibility to mood disorders. Future studies will investigate the neurobiological mechanisms that underlie the sex-specific processing of abusive stimuli. Research Support: This research was supported by a seed grant from the Brains and Behavior program at Georgia State University.

12.00-14.00 Conference Symposium 2: THE IMPACT OF STRESS ON HIPPOCAMPAL PLASTICITY AND MEMORY: LINKING ELECTROPHYSIOLOGY TO BEHAVIOR
Chairs: NB Yeritsyan, JU Frey (presentations 25 min)

- INTRODUCTION
- HJ Krugers (Amsterdam, The Netherlands) STRESS-HORMONES, SYNAPTIC PLASTICITY AND MEMORY FORMATION
- JG Howland, BN Cazakoff, MJ MacDougall (Saskatchewan, Canada) EFFECTS OF ACUTE STRESS ON SHORT- AND LONG-TERM SYNAPTIC PLASTICITY IN THE HIPPOCAMPAL CA1 AND SUBICULAR REGIONS
- NB Yeritsyan, JU Frey (Magdeburg, Germany) SWIM STRESS ALTERS HIPPOCAMPAL PLASTICITY IN REGION-SPECIFIC MANNER: STRESS-INDUCED SYNAPTIC CROSS-TAGGING IN THE CA1 AND EXCITABILITY CHANGES IN THE DENTATE GYRUS
- DM Diamond (Tampa, USA) TRANSLATIONAL RESEARCH FROM SYNAPTIC PLASTICITY TO CLINICAL OBSERVATIONS PROVIDES INSIGHT INTO THE NEUROBIOLOGY OF FLASHBULB AND TRAUMATIC MEMORIES

INTRODUCTION: THE IMPACT OF STRESS ON HIPPOCAMPAL PLASTICITY AND MEMORY: LINKING ELECTROPHYSIOLOGY TO BEHAVIOR. NB Yeritsyan, JU Frey, Department of Neurophysiology, Leibniz Institute for Neurobiology, Center of Learning and Memory Research, Magdeburg, Germany

Stress has long been recognized to strongly modulate learning and memory. With respect to hippocampus-dependent learning stress has been reported to either facilitate or block acquisition, consolidation, and/or recall of relevant memory tasks, depending on experimental conditions. The exact mechanisms of such diverse effects are still an object of burning interest and discussion. The ample evidence, that stress differentially influences LTP/LTD in the hippocampus (synaptic plasticity phenomena widely used as cellular models to study mechanisms of memory formation), suggests a testable path to study memory deficits triggered by stressful experience. In particular, if changes of synaptic plasticity are essentially involved in learning and memory processes, then stress-associated LTP/LTD alteration might also constitute a neuronal basis for stress-induced memory impairment. This symposium will provide a closer look on the candidate mechanisms by which stress modulates hippocampal functional plasticity and hippocampus-dependent learning. New insights at different investigation levels will be presented by studies of stress- and stress-hormone-induced effects on synaptic plasticity and/or on neuronal excitability in the hippocampus. Further, plasticity changes and learning deficits triggered by stress will be discussed with regard to functional diversity and the physiological significance when comparing ventral with dorsal parts of the hippocampus and its subregions CA1, CA3 and DG. The role of neuromodulatory inputs from other brain structures (such as the basolateral amygdala, ventral tegmental area, etc.) in shaping the region-specific pattern of hippocampal plasticity and in the outcome of hippocampus-dependent learning tasks under stress condition will be discussed. This session will demonstrate the critical importance of understanding the mechanisms of stress-altered hippocampal plasticity and memory in behavioral disorders precipitated by stress, for instance, post-traumatic stress disorder (PTSD).

STRESS-HORMONES, SYNAPTIC PLASTICITY AND MEMORY FORMATION. HJ Krugers, University of Amsterdam, Amsterdam, The Netherlands

Humans and rodents retain memories for emotionally arousing and stressful events very well. The facilitated retention of these memories is normally very useful: individuals can appraise and if necessary avoid similar negative situations in future. The memory enhancing effects of stress are mediated by hormones, such as norepinephrine and glucocorticoids which are released during stressful experiences. An important question is how these hormones facilitate cognitive processes. Synaptic transmission and plasticity of excitatory synapses is not only critical for proper development of the central nervous system, but forms also the fundamental basis for the ability to store information in the brain. Recent studies have demonstrated that glucocorticoids promote excitatory synaptic transmission in the brain by increasing trafficking and synaptic insertion of AMPA type glutamate receptors. These effects are slowly induced, mediated by glucocorticoid receptors and require protein synthesis. At the same time that these hormones increase synaptic strength, they also prevent the activity-dependent increase in synaptic AMPA receptors and affect synaptic plasticity. We are currently exploring how these hormones regulate i) trafficking and ii) synaptic insertion of AMPA receptors, iii) if glucocorticoids interact with other stress-hormones to modulate synaptic transmission and plasticity, and iv) if regulation of AMPA receptors underlies the memory enhancing effects of stress-hormones.

EFFECTS OF ACUTE STRESS ON SHORT- AND LONG-TERM SYNAPTIC PLASTICITY IN THE HIPPOCAMPAL CA1 AND SUBICULAR REGIONS. JG Howland, BN Cazakoff, MJ MacDougall, Departments of Physiology and Psychology, University of Saskatchewan, Saskatchewan, Canada

Acute stress has complex effects on hippocampal dependent forms of learning and memory. The physiological basis of these effects may include dynamic changes in hippocampal synaptic plasticity caused by acute stress. The present experiments assessed the effects of acute stress on patterns of both short- and long-term synaptic plasticity in the dorsal hippocampal CA1 and subicular regions of anesthetised rats. Adult male Sprague Dawley rats were anesthetised with urethane and secured in a stereotaxic apparatus. Bipolar stimulating and monopolar recording electrodes were slowly lowered into either then CA3-CA1 or the CA1-dorsal subiculum regions of the dorsal hippocampus. Short-term plasticity was assessed using paired pulse facilitation (PPF; 25, 50, 100, and 200 ms intervals). Long-term plasticity was assessed with both high (100 and 200 Hz) and low frequency (1 and 3 Hz) tetanus protocols. In some animals, acute stress was induced by exposure to an elevated platform (30 min) and glucocorticoid receptors (GRs) were blocked using the antagonist RU38486 (10 mg/kg). PPF was observed in the CA1 and subicular regions of control animals. Acute stress significantly reduced PPF in the CA1, but not subiculum, through activation of GRs. Patterns of long-term synaptic plasticity differed in the CA1 and subiculum. While high frequency stimulation induced significant long-term potentiation (LTP) in both regions of control animals, low frequency stimulation had no effect in the CA1 region and induced a slow developing potentiation in the subiculum. Acute stress blocked both LTP and the slow developing potentiation in the subiculum, effects that depended on glucocorticoid receptor activation. These results demonstrate regionally specific effects of acute stress on plasticity in the hippocampal formation and may provide insight into the mechanisms underlying the effects of acute stress on learning and memory. Research Support: This work was supported by a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada awarded to JGH.

SWIM STRESS ALTERS HIPPOCAMPAL PLASTICITY IN REGION-SPECIFIC MANNER: STRESS-INDUCED SYNAPTIC CROSS-TAGGING IN THE CA1 AND EXCITABILITY CHANGES IN THE DENTATE GYRUS. NB Yeritsyan, JU Frey, Department of Neurophysiology, Leibniz Institute of Neurobiology, Center of Learning and Memory Research, Magdeburg, Germany

An alteration of hippocampal functional plasticity by stress is thought to represent a mechanism, which underlies stress-induced memory impairment. Diverse effects of stress on hippocampal LTP (*in vitro* and *in vivo*) could be caused by differences in the experimental stress-models, stimulation protocols, the locus of stimulation (e.g., ventral vs. dorsal parts of the hippocampus and hippocampal subregions (CA1, CA3 or dentate gyrus, DG). Recent studies spread light on pathways and the physiological significance of stress-associated changes in ventral and dorsal portions of the hippocampus. However, the exact mechanisms as well as the potential of hippocampal synaptic plasticity shaped by stress in a region-specific manner remain to be clarified. To this end, we have investigated the effect of swim stress on LTP in the DG and CA1 region of the dorsal hippocampus. An improved technique of extracellular field recordings has been used in freely moving adult rats to simultaneously record the field excitatory postsynaptic potential (fEPSP) and the population spike at their places of generation in medial perforant path-DG- and in commissural path-CA1-synapses. Our findings suggest that swim stress modulates functional plasticity in the DG rather through excitability changes than through synaptic events. In contrast, stress alters CA1-LTP at the synaptic level similar to the model of synaptic 'cross-tagging' (for review see Frey and Frey, 2008) by transforming E-LTP or L-LTP into L-LTD. Further, the impact of neuromodulatory inputs from the ventral tegmental area (VTA), as well as the effects of the dopaminergic D2-receptor antagonist eticlopride or the MEK inhibitor U0126 on basal synaptic transmission and heterosynaptic LTP in the CA1 have been approached under swim stress and non-stress conditions. It has been shown that the timing of the swim episode in relation to tetanization as well as VTA-activation and/or administration of eticlopride or U0126 within distinct times around electrophysiological or behavioral procedures is important to determine the associative plasticity outcome in the CA1. Altogether, these results assured a synaptic nature of stress-triggered plasticity in the CA1 region and proved that swim stress specifically modulates functional plasticity in hippocampal subregions, whereas the mechanisms in CA1 and DG are different.

TRANSLATIONAL RESEARCH FROM SYNAPTIC PLASTICITY TO CLINICAL OBSERVATIONS PROVIDES INSIGHT INTO THE NEUROBIOLOGY OF FLASHBULB AND TRAUMATIC MEMORIES. DM Diamond, Department of Psychology, University of South Florida and Research and Development Service, Tampa Veterans Hospital, Tampa, FL, USA

It is well-known that the hippocampus is necessary for the formation of new memories. However, research on rodents and humans indicates that in times of stress hippocampal functioning is inhibited. This

finding has been interpreted to indicate that there is an absence of hippocampal involvement in the formation of traumatic memories. I will incorporate findings from rodent and human studies to provide an alternative interpretation of the literature. I will suggest that traumatic experiences produce a relatively brief and intense activation of amygdala-hippocampal circuitry, with a concomitant suppression of neocortical functioning, which helps to explain the unique and fragmentary nature of traumatic memories. This perspective on how stress affects the hippocampus, amygdala and neocortex is potentially relevant toward understanding how traumatic experiences generate long-lasting intrusive memories which are highly resistant to extinction.

15.00-15:30 Conference Lecture:
REDUCED FORM COQ10 RESCUED THE SOCIAL BEHAVIOR DEFICIT IN CHICK DURING DEVELOPMENT. S Nakamura, S Ozawa, S Obara, G Karino, H Sekihara, Y Fukushima, Y Shirakawa, K Mimura, K Fuji, M Koshiba, Tokyo University of Agriculture and Technology, Department of Biotechnology and Life Science, Tokyo, Japan

We have developed the social affiliation animal model using domestic chick and marmoset. The development of the social behavior proceeded through several critical periods which may be regulated by monoaminergic nervous systems. The early limbic monoaminergic system is susceptible to the oxidative stress. Here we introduced reduced form CoQ10 to our chick model as a fuel for energy production organelle and neuroprotective agent as well and examined its effect on the social behavior deficit. The multivariate analysis algorism to visualize the correlation structure of social behavior and physiological parameters was successfully applied in this experiment. Reduced form CoQ10 (KANEKA Corp., Osaka) was orally supplied as pure corn oil-CoQ10-mixed food pellets daily at 20-1200mg/head during day 5-21(P21) after hatching. The serum level CoQ10 was measured by HPLC with P21 old chick. Only socially isolated chick was supplied with rCoQ10 and experienced mutual interaction with socially grouped chicks for 10 min per day during P13-15. The control chick was socially isolated and experienced the mutual interaction with oil-mixed food pellets. The behavior was tested at P13, P16, and P21 and was analyzed by principal component analysis (PCA). The serum level rCoQ10 reached more than 3 times over control chick level at 200mg/head. The behavior development during P13-P21 was compared between rCoQ10 chick and without-chick in parallel with typical socially-grouped and -isolated chicks. The rCoQ10 chick changed its behavior rather similar to the grouped chick in approaching behavior. No rCoQ10 chick behaved similar to the socially isolated chick at P21. This result suggested that food supplement other than antipsychotic drug may help social adaptation. Research Support: MEXT Grant-in-Aid for Scientific Research on Innovative Areas 21200017, JST895251, AS2211728E, 165002202.

15.30-18.00 Conference Symposium 3: TUNA NEUROSCIENCE SYMPOSIUM: EXPERIMENTAL MODELS IN STRESS RESEARCH
Chairs: AV Kalueff, JE Warnick, TUNA (presentations 20 min)

- INTRODUCTION: TULANE UNIVERSITY NEUROSCIENCE ASSOCIATION (TUNA)
- DJ Guarnieri, CE Brayton, SM Gray, J Maldonado-Aviles, JR Trinko, J Nelson, JR Taylor, SL Gourley, RJ DiLeone (New Haven, USA) GENE PROFILING REVEALS A ROLE FOR STRESS HORMONES IN THE MOLECULAR AND BEHAVIORAL RESPONSE TO FOOD RESTRICTION
- J Halonen, P Zoladz, DM Diamond (Tampa, USA) NEUROBIOLOGY OF FORGOTTEN BABY SYNDROME
- B Bobula, A Gadek-Michalska, G Hess (Krakow, Poland) EFFECTS OF RESTRAINT STRESS ON LONG-TERM POTENTIATION IN RAT FRONTAL CORTEX
- J Wabno, G Hess (Krakow, Poland) EFFECTS OF REPEATED CORTICOSTERONE ADMINISTRATION ON GLUTAMATERGIC AND GABAERGIC TRANSMISSION IN RAT FRONTAL CORTEX
- T Kuwaki (Kagoshima, Japan) A PIVOTAL ROLE OF OREXIN (HYPOCRETIN) NEURONS IN FIGHT-OR-FLIGHT RESPONSE
- A Gądek-Michalska, M Szymańska, J Tadeusz, J Spyrka, P Rachwalska, J Bugajski (Krakow, Poland) EFFECT OF REPEATED RESTRAINT STRESS ON IL-1b IN PLASMA AND BRAIN STRUCTURES AND HPA AXIS RESPONSE
- M Koshiba, A Senoo, S Obara, K Mmimura, Y Shirakawa, G Karino, K Otsuhata, M Takahashi, K Yui, H Yamanouchi, S Nakamura (Tokyo, Japan) AN EMOTIONAL MENTAL STATE EXPRESSED AS A CORRELATION STRUCTURE BETWEEN BEHAVIOR AND PHYSIOLOGICAL PARAMETERS BEYOND SPECIES DIFFERENCE

GENE PROFILING REVEALS A ROLE FOR STRESS HORMONES IN THE MOLECULAR AND BEHAVIORAL RESPONSE TO FOOD RESTRICTION. DJ Guarnieri, CE Brayton, SM Gray, J Maldonado-Aviles, JR Trinko, J Nelson, JR Taylor, SL Gourley, RJ DiLeone, Division of Molecular Psychiatry, Ribicoff Research Facilities, Department of Psychiatry, Yale University School of
Medicine, New Haven, CT, USA

Food restriction is known to enhance learning and motivation. These changes likely involve alterations in gene expression in brain regions mediating behavioral flexibility and motivation. Assessment of gene expression profiles in male C57BL6/J mice using whole-genome microarrays was completed in the medial prefrontal cortex, nucleus accumbens, ventral tegmental area, and the hypothalamus following a brief food restriction. Quantitative PCR was used to validate these findings in independent samples. In situ analysis was performed to characterize expression profiles in the brain. Plasma levels of the stress hormone corticosterone (CORT) were measured by ELISA. Expression changes were measured in adrenalectomized animals that underwent food restriction, as well as in animals receiving daily injections of CORT. Progressive ratio responding for food, a measure of motivated behavior, was assessed after CORT treatment in restricted and fed animals.

Results and Discussion: Brief food restriction results in an up regulation of peripheral stress responsive genes in the mammalian brain. Time-course analysis demonstrated rapid and persistent expression changes in all four brain regions under study. Administration of CORT to non-restricted animals was sufficient to induce a subset of the genes, and alterations in gene expression after food restriction were dependent on intact adrenal glands. CORT can increase the motivation to work for food only in the restricted state. These data demonstrate a central role for CORT in mediating both molecular and behavioral responses to food restriction. The stress hormone-induced alterations in gene expression described here may be relevant for both adaptive and pathological responses to stress. Research Support: This work was supported by UL-DE19586 and RL1AA017537 well by the State of Connecticut, Department of Mental Health and Addiction Services (RJD and JRT).

NEUROBIOLOGY OF FORGOTTEN BABY SYNDROME. J Halonen, P Zoladz; DM Diamond, VA Hospital Tampa, Departments of Psychology and Pharmacology, University of South Florida, Tampa, FL; Department of Psychology, Ohio Northern University, Ada, OH, USA

Forgotten Baby Syndrome (FBS) occurs when a parent or caretaker exits a car and in the process, completely forgets that a child in his or her care is still within the car. This incomprehensible lapse of memory exposes forgotten children to lethal hazards, including death from hyperthermia caused by confinement of the child in a hot car. Based on information provided by the media and http://www.kidsandcars.org, at least 200 children in the past 15 years have died of FBS in the U.S., with reports of fatal FBS occurring worldwide. How can normal, loving and attentive parents, with no evidence of substance abuse or an organic brain disorder, have a lapse of memory which results in the death of a child? We have developed a two-part hypothesis to address the etiological basis of FBS. First, we have evaluated whether there is a consistent pattern of circumstances that may provide insight into occurrences of FBS. Second, we have speculated on the neurobiological substrates that underlie FBS. We hypothesize that FBS is a result of a catastrophic interaction of habit and prospective forms of memory. Prospective memory (PM) is the planning and execution of an action to take place in the future; forgetting to drop off a child at daycare on the way to work is an example of a failure of PM. We have found that in cases of FBS the parents were typically engaged in a well-developed routine which did not include taking the child to daycare (habit memory; HM). We hypothesize that the performance of the HM, e.g. drive straight from home to work, interferes with PM-based processing. A secondary feature of a subset of cases of FBS is that parents experienced impaired sleep the night before the FBS event, and/or they experienced a powerful stressor during the drive. We propose that the combination of sleep deprivation, stress and HM interfered with the activation of the memory to interrupt the routine and, instead, bring the child to the daycare provider. We further hypothesize that the neurobiological substrates of FBS involve 4 primary brain structures: The prefrontal cortex (PFC), hippocampus, amygdala and basal ganglia (BG). In theory, the PFC and hippocampus underlie episodic, PM processing, which can be suppressed by habit-based functioning of the BG, which acts alone, or in conjunction with stress-induced activation of the amygdala.

EFFECTS OF RESTRAINT STRESS ON LONG-TERM POTENTIATION IN RAT FRONTAL CORTEX. B Bobula, A Gadek-Michalska, G Hess, Department of Physiology, Institute of Pharmacology PAN, Krakow, Poland

Chronic stress and resulting from it, prolonged hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis and elevated level of glucocorticoids in the circulatory system, have been linked to the

pathophysiology of depressive disorders. Depressive disorders are associated with structural and functional abnormalities in a number of brain structures including frontal cortical areas. Long-term potentiation (LTP) is widely regarded as a model of synaptic plastic processes underlying learning and memory. Many studies have shown that various forms of behavioral stress inhibit LTP in rat hippocampus. In contrast, little is known about the influence of stress on LTP in the neocortex. Here we sought to determine the effects of repetitive restraint on LTP induction in rat frontal cortex ex vivo. Methods: Male young adult rats were subjected to restraint in metal tubes lasting for 10 min (2 times daily, repeated for 3 consecutive days). Control animals were kept in home cages. 24 h after last stress session rats were decapitated, their brains were removed and frontal cortical slices (420 µm thick) were cut in the coronal plane using a vibrating microtome. Field potentials were evoked in cortical layer II/III by stimulation of underlying sites in layer V. Results and Discussion: The analysis of the relationship between stimulation intensity and the response magnitude revealed no significant differences between slices prepared from naïve and stressed animals over the whole range of stimulation intensities. LTP was induced by the series of theta burst stimulations (TBS). In slices obtained from stressed rats the magnitude of LTP, measured 1 hour after TBS, was decreased in comparison to that in slices obtained from control animals (115 ± 6 % vs 141 ± 6,5 % of baseline; $P<0.001$; $F(1160,899)$, one-way ANOVA with post hoc Tukey test). These data indicate that short repeated restraint stress does not influence basal synaptic transmission in rat ex vivo frontal cortex. However, it decreases the potential for long-term synaptic plasticity. Research Support: This work was supported by European Union: Grant POIG 01.01.02–12-004/09-00 “Depression-Mechanisms-Therapy” awarded by the European Regional Development Fund and and by the statutory activity of the Institute of Pharmacology, Polish Academy of Sciences, Krakow.

EFFECTS OF REPEATED CORTICOSTERONE ADMINISTRATION ON GLUTAMATERGIC AND GABAERGIC TRANSMISSION IN RAT FRONTAL CORTEX. J Wabno, G Hess. Department of Physiology, Institute of Pharmacology PAN, Jagiellonian University, Krakow, Poland

Chronic stress and related to it, prolonged hyperactivation of the hypothalamic-pituitary-adrenal axis and exposure to high level of glucocorticoids in the circulatory system, have been linked to the pathophysiology of depressive disorders. Alterations in the functions of the main excitatory (glutamatergic) and inhibitory (GABAergic) neurotransmitter systems of the central nervous system have been suggested to be involved in the pathophysiology of depression. Repeated corticosterone administration represents an animal model to study the effects of non-adaptative stress. The aim of the present study was to examine the influence of repeated corticosterone administration on excitatory and inhibitory synaptic transmission in ex vivo slices of the frontal cortex. Male Wistar rats were treated with corticosterone (10 mg/kg s.c.; suspended in 1% Tween 80) twice daily, for 7, 14 or 21 days. Frontal cortical slices were prepared 48 hours after last drug administration. Whole-cell recordings of spontaneous excitatory postsynaptic currents (sEPSCs) and spontaneous inhibitory postsynaptic currents (sIPSCs) were made from layer II/III pyramidal cells at the holding membrane potential of -78 mV and 0 mV, respectively. The frequency, the amplitude, the rise time and decay time constant of sEPSCs and sIPSCs were measured. Results and Discussion: In slices prepared from animals treated with corticosterone for 7, 14 or 21 days we observed an increase of the mean sEPSCs frequency but not the mean sEPSCs amplitude. In slices prepared from rats treated with corticosterone for 7 days, but not for 14 and 21 days, a decrease of the rise time and of decay time constant of sEPSCs were detected. In contrast, no corticosterone-induced changes in parameters characterizing sIPSCs were evident. These data demonstrate that repeated corticosterone treatment affects glutamatergic transmission leaving GABAergic transmission intact. Research Support: Supported by the Ministry of Science and Higher Education, Warsaw, Poland (NN401 000137).

A PIVOTAL ROLE OF OREXIN (HYPOCRETIN) NEURONS IN FIGHT-OR-FLIGHT RESPONSE. T Kuwaki, Department of Physiology, Graduate School of Medical and Dental Sciences, Kagoshima University, Japan

Stress increases cardiac function, ventilation, and body temperature. These changes prepare the body for fight-or-flight behavior by increasing the metabolic rate, oxygen supply, and by conduction velocity of nerve impulses. A key role of subregion of the hypothalamus, so called “defense area”, has long been known but precise mechanisms and/or subserving neurotransmitters were largely known. Our recent research results shed light on orexin (hypocretin) neurons as a master switch which triggers multiple components of the fight-or-flight (defense) response (Kuwaki and Zhang, Resp Physiol Neurobiol 174: 43-54, 2010). As an extension, we thought that the stress-induced hyperthermia would also depend on orexin. We used handling stress model in which a temperature probe was repetitively inserted into the animal’s rectum. Results and Discussion: We found, contrary to our expectations, orexin neuron-ablated mice (ORX-AB) but not orexin knockout mice (ORX-KO) have a blunted stress-induced hyperthermia. The brown adipose tissue, which is a major thermogenic organ in rodents, did not respond to handling stress although it did respond to a direct

pharmacologic stimulation. These abnormalities in ORX-AB were not observed in ORX-KO in which orexin peptide is deficient but neurons are preserved. In wild-type mice and ORX-KO, handling stress activated orexin neurons (as revealed by increased expression of c-Fos) and the resultant hyperthermia was largely blunted by pretreatment with a β3 antagonist. This observation further supports the notion that attenuated stress-induced hyperthermia in ORX-AB mice was caused by a loss of orexin neurons and abnormal BAT regulation. Therefore, integrity (orexin and co-existing other neurotransmitter/modulators) of the orexin neurons is indispensable for full expression of multiple facets of the fight-or-flight response. Research Support: Part of the work was supported by the Grants-in Aid for Scientific Research from the Ministry of Education, Science, Culture and Sports, Japan.

EFFECT OF REPEATED RESTRAINT STRESS ON IL-1ß IN PLASMA AND BRAIN STRUCTURES AND HPA AXIS RESPONSE. A Gądek-Michalska, M Szymańska, J Tadeusz, J Spyrka, P Rachwalska, J Bugajski, Department of Physiology, Institute of Pharmacology, Krakow, Poland

Pro-inflammatory cytokine interleukin-1ß (IL-1ß) level is modulated during stress reactions in brain structures involved in hypothalamic-pituitary-adrenal axis (HPA) regulation. Multiple stressors induce different IL-1ß and HPA responses. The purpose of the present study was to determine if the effect of prior repeated restraint stress on IL-1ß levels in prefrontal cortex, hippocampus, hypothalamus and plasma may have an impact on contemporary induced alterations in HPA-axis responses. Adult male Wistar rats were exposed to 10 min restraint stress twice a day for 3 days. 24 h after the last stress period rats were injected i.p. with a single dose of IL-1ß (1 µg/rat), IL-1ß receptor antagonist or saline. After rapid decapitation, trunk blood for plasma determinations and prefrontal cortex, hippocampus and hypothalamus were excised and frozen at -70 ℃. Total IL-1ß, ACTH and corticosterone levels were determined in plasma using commercially available kits. Western blot analyses were performed on brain structures samples. In non-stressed rats exogenous IL-1ß significantly increased plasma ACTH and corticosterone levels in a time dependent manner, more strongly at 2 h than 1 h after IL-1ß administration. By contrast plasma IL-1ß levels were more potently augmented 1h than after 2 h following injection suggesting that the increase in plasma IL-1ß levels precedes stimulation of ACTH and corticosterone secretion. Repeated restraint substantially augmented the resting plasma levels of both IL-1ß, ACTH and corticosterone 24 h after the last restraint. Prior restraint intensified the plasma IL-1ß, ACTH and corticosterone responses to IL-1ß 1 h following administration. Pretreatment with IL-1ß antagonist abolished the increase in ACTH and corticosterone responses to IL-1ß induced by both repeated stress alone and combined with exogenous IL-1ß administration. These results suggest that repeated stress increases IL-1ß production which activates ACTH and corticosterone secretion and sensitizes these responses to exogenous IL-1ß. Repeated stress markedly increased IL-1ß level in brain structures involved in HPA axis regulation and intensified this increase evoked by exogenous IL-1ß. These results support the role of brain and peripheral IL-1ß in adaption of HPA response during prolonged stress. This research was supported by a grant POIG 01.01.02-12-004/09-00 ”Depression-Mechanisms-Therapy” financed by European Regional Development Fund.

AN EMOTIONAL MENTAL STATE EXPRESSED AS A CORRELATION STRUCTURE BETWEEN BEHAVIOR AND PHYSIOLOGICAL PARAMETERS BEYOND SPECIES DIFFERENCE. M Koshiba, A Senoo, S Obara, K Mmimura, Y Shirakawa, G Karino, K Otsuhata, M Takahashi, K Yui, H Yamanouchi, S Nakamura, Tokyo University of Agriculture and Technology, Jizaiken, Tsukiminosono, Ashiya University, Saitama Medical University, Japan

We have developed the multivariate analysis algorism to visualize the correlation structure of social behavior and physiological parameters during well-defined context of social interaction mainly based on animal models (chick and marmoset). Now, we apply the algorism to reveal the human mental state as a correlation structure between the objective parameters like behavior and physiological data and the subjective psychological scales. In view of the oxidative stress impact on the neuronal systems through aging process, we would like to introduce antioxidant dietary supplement as chronic metal care. The research protocol was approved by the human research committee of Tokyo University of Agriculture and Technology. The behavior during pediatric treatment, private cram school lesson for learning disability children or physical exercise at a nursing home for the aged was video recorded and monitored by infrared camera (CHINO Cor., Tokyo). Physiological parameters like EEG, heartbeat, body surface temperature (Intercross Ltd., Tokyo) and SpO2 (Konica Minolta Inc., Tokyo) as well as serum level cortisol etc. were monitored. The correlation of behavior, physiological parameters, and psychological interview scale were analyzed by principal component analysis (PCA). Results and Discussion: In the juvenile studies for developmental disorders, both individual- and group-specific patterns were expressed differently as their contextual-dependent variances in the projection plane after PCA. The variance patterns were preliminarily confirmed to be reproducible in time course studies.

In elderly studies, the correlative structures between psychological scores and serum markers were more complex, suggesting how matured each mental state is. Research Support: MEXT Grant-in-Aid for Scientific Research on Innovative Areas 21200017, JST895251, AS2211728E, 165002202.

18.00-18.45 Conference Presentation:
CLEVER SYS BEHAVIOR RECOGNITION TECHNOLOGY AND HOME CAGE BEHAVIOR ANALYSIS. V Kobla, Y Liang, Clever Sys Inc, Reston, VA, USA

Clever Sys Inc is a leading provider of Automated Behavior Analysis systems based on the proprietary patent-protected Behavior Recognition technology. This technology enables automated analysis of complex, natural, and stereotypic behaviors that relies on the analysis of the full animal body and the identification of body parts, and modeling behaviors based on the movement patterns of the full animal body and its body parts. Products such as HomeCageScan, TopScan, & GroupHousedScan provide state-of-the-art platforms for monitoring behavior activity of individual and group-housed animals in their natural environments with minimal human intervention. All products have the capability to operate in real-time and in high-throughput modes, thereby allowing large numbers of animals to be screened in an efficient manner. Our products can provide both quick screening capabilities as well as comprehensive and in-depth analysis of specific behavior paradigms. Home Cage Behavior analysis is of emerging importance in the field of behavior analysis, as tools such as HomeCageScan get adopted into more widespread use. We highlight the salient features of HomeCageScan and its application in various fields of research, as well as future trends in behavior analysis.

18.45-19.20 Conference Presentation:
SCHOLARLY VIDEO PUBLISHING TO INCREASE PRODUCTIVITY AND STANDARDIZATION IN MEDICAL AND LIFE SCIENCES. L Colbert, Journal of Visualized Experiments, Somerville, USA

The world of academic publishing has remained relatively unchanged since the first scientific journal in 1665. The Royal Society believed that science could only move forward through a transparent and open exchange of ideas backed by experimental evidence, and it was on this belief that the first English journal was built. It took over a hundred years for the next innovation of colour lithography to appear, and since then the most important advancements made in scientific publishing have been centuries apart. The creation of the Internet in the twentieth century brought about another significant change in the approach of ePublishing, which allowed for easier accessibility and a more rapid transfer of knowledge. In 2006, the Journal of Visualized Experiments, JoVE, introduced a new format of publishing that had a significantly higher capacity for transparency and visualisation than traditional print media of the past. This novel, multimedia format allows for an increase in the efficacy of knowledge transfer through the dissemination, discussion and reproduction of experimental approaches in a visual blueprint. During this session, we will discuss the limitations that scientists and clinicians have faced in advancing research through publication and how JoVE has addressed these issues through continuous innovation in formatting and technology. The journal remains committed to maintaining the scientific integrity and ethical standards of methodological research and applied techniques as it evolves to meet the changing needs of the research community. Originally founded for the publication of basic biological life science methodologies, JoVE has expanded to cover multidisciplinary approaches of research in neuroscience, immunology and infectious disease, bioengineering, and translational medicine. We are further expanding to offer the same transfer of knowledge to the clinical community. JoVE is the first and only peer reviewed protocols journal dedicated to publishing techniques in visual format. The journal has currently published over 1000 video articles demonstrating the methods and techniques used to advance modern scientific research.

Day 3. June 24, 2011

Main Auditorium, Tulane University Medical School (1430 Tulane Avenue), Downtown Campus, New Orleans, LA, USA

9.00-9.30 Conference Presentation:
JOURNAL OF VISIALIZED EXPERIMENTS: INCREASING STANDARDIZATION AND IMPROVING EDUCATION IN BIOMEDICAL SCIENCES. L Colbert, Journal of Visualized Experiments, Somerville, USA

9.30-11.30 Conference Symposium 4: ZEBRAFISH NEUROSCIENCE SYMPOSIUM – ZEBRAFISH NEUROSCIENCE RESEARCH CONSORTIUM SYMPOSIUM
Chairs: M Viana, C Bonan (presentations 1-2: 30 min, presentations 3-4: 30 min)

- INTRODUCTION
- AL Piato, G Ghisleni, DB Rosemberg, KM Capiotti, AM Siebel, AP Herrmann, MR Vianna, MR Bogo, DR Lara, CD Bonan (Porto Alegre, Brazil) MODELING ACUTE RESTRAINT STRESS PROTOCOL IN ZEBRAFISH (DANIO RERIO): IMPLICATIONS ON BEHAVIORAL AND PURINERGIC SIGNALING PARAMETERS
- MR Vianna, AL Piato, KM Capiotti, AR Tamborski, JP Oses, LJ Barcellos, MR Bogo, DR Lara, CD Bonan (Porto Alegre, Brazil) PHYSIOLOGICAL AND BEHAVIORAL IMPACTS OF AN UNPREDICTABLE CHRONIC STRESS MODEL IN ZEBRAFISH
- AV Kalueff, E Kyzar, I Zapolsky, S Gaikwad, A Roth, J Green, M Pham, PG St. Pierre, B Hirons (New Orleans, USA) ZEBRAFISH NEUROPHENOME PROJECT
- JE Warnick, A Linker (Russellville, USA) THE ASSESSMENT OF MAXIMUM PREDICTIVE VALUE IN A ZEBRAFISH ANXIOLYTIC SCREENING ASSAY

MODELING ACUTE RESTRAINT STRESS PROTOCOL IN ZEBRAFISH (DANIO RERIO): IMPLICATIONS ON BEHAVIORAL AND PURINERGIC SIGNALING PARAMETERS. ÂL Piato, G Ghisleni, DB Rosemberg, KM Capiotti, AM Siebel, AP Herrmann, MR Vianna, MR Bogo, DR Lara, CD Bonan. Pontifícia Universidade Católica do Rio Grande do Sul, Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil

Exposure to stressors are able to induce changes in the concentrations of extracellular signaling molecules related to purinergic system in mammals, such as ATP and adenosine. In addition, changes in activities of enzymes involved in nucleotide hydrolysis have also been reported in the rat spinal cord and blood serum after repeated restraint stress. Despite the extensive knowledge about the effects of acute restraint stress in rodents, zebrafish research is still elementary in this field, and the neurobiological underlying mechanisms and consequences of stress on purinergic system are particularly unclear. Therefore, here we evaluated the effects of acute stress on behavioral, biochemical, and molecular parameters of purinergic signaling in zebrafish. Methods: Groups of animals were submitted at different time periods of restraint stress (15, 45, 60 or 90 min) to protocol standardization. Since crf gene expression was altered only after 90 min of confinement, the experiments related to anxiety levels, exploratory behavior, and memory performance were performed using this time period, which also showed an increase in whole-body cortisol levels. We analyzed the activity and gene expression of enzymes involved in the control of nucleotide and nucleoside levels (NTPDases, 5'-nucleotidase, and adenosine deaminase) as well as the transcription of adenosine receptors subtypes and corticotropin-releasing factor. Results and Discussion: The acute restraint stress protocol increased anxiety, but did not impair locomotion or cognitive function. We verified that whole-body cortisol levels presented a 46% increase at 90 min of restraint stress in relation to control animals. However, the expression patterns of brain CRF mRNA showed a 53% reduction at 90 minutes of restraint stress in relation to control group. Regarding the purinergic system, the results showed that acute restraint stress significantly increased ATP hydrolysis (47%), whereas both ADP and AMP hydrolysis were not altered. Moreover, the stress protocol promoted a significant decrease (29%)on cytosolic ADA activity, while ecto-ADA activity was not changed. RT-PCR experiments showed that the relative entpd1, entpd2, and entpd2a.1 gene expression significantly decreased after 90 min of stress protocol. However, entpd2a.2, entpd3, nt5e, and ada1 gene expression profiles remained unaltered. The evaluation of adenosine receptors in zebrafish brain demonstrated that adora1, adora2a.1, adora2a.2, and adora2b mRNA levels significantly increased after the stress protocol. The net charge of the alterations here observed is likely an increase in adenosinergic transmission. We observed that acute restraint stress disturbed zebrafish behavior and hypothesize that the augmentation in adenosine-mediated signaling may be part of the allostatic responses of the organism as an attempt to reestablish homeostasis and normal behavior after a stressful event. Research Support: DECIT/SCTIE-MS through CNPq and FAPERGS (Proc. 10/0036-5–PRONEX/Conv. 700545/2008), CAPES, CNPq,INCT-TM.

PHYSIOLOGICAL AND BEHAVIORAL IMPACTS OF AN UNPREDICTABLE CHRONIC STRESS MODEL IN ZEBRAFISH. MR Vianna, AL Piato, KM Capiotti, AR Tamborski, JP Oses, LJ Barcellos, MR Bogo, DR Lara, CD Bonan. Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brasil

Zebrafish (Danio rerio) have emerged as a promising model organism to study development, toxicology, pharmacology, and neuroscience, among other areas. Despite the increasing number of studies using zebrafish, behavioral studies with this species are still elementary when compared to rodents. The aim of this study was to develop a model of unpredictable chronic stress (UCS) in zebrafish. Methods: We

evaluated the effects of a novel UCS protocol lasting 7 or 14days on behavioral and physiological parameters. The effects of stress were evaluated in relation to anxiety and exploratory behavior, long-term inhibitory avoidance memory, expression of corticotrophin-releasing factor (CRF) and glucocorticoid receptor (GR), and cortisol levels. Results and Discussion: As expected, UCS protocol increased the anxiety levels, impaired cognitive function, and increased CRF while decreased GR expression. Moreover, zebrafish submitted to 7 or 14days of UCS protocol presented increased cortisol levels. The protocol developed here is a complementary model for studying the neurobiology and the effects of chronic stress in behavioral and physiological parameters. In addition, this protocol is less time consuming than standard rodent models commonly used to study chronic stress. These results confirm UCS in zebrafish as an adequate model to preclinical studies of stress, although further studies are warranted to determine its predictive validity. Research Support: DECIT/SCTIE-MS through CNPq and FAPERGS (Proc. 10/0036-5–PRONEX/Conv. 700545/2008) and CNPq 567493/2008-8.

ZEBRAFISH NEUROPHENOME PROJECT. AV Kalueff, E Kyzar, I Zapolsky, S Gaikwad, A Roth, J Green, M Pham, P St-Pierre, B Hirons. Tulane University Medical School, Department of Pharmacology and Neuroscience Program, Tulane Neurophenotyping Platform, Innovative Learning Center, Technology Services, New Orleans, LA, USA

Zebrafish *(Danio rerio)* have been widely used in recent neuroscience research, offering researchers a novel model organism to identify genetic and environmental factors involved in the regulation of various brain functions. Recent advances in information technology have allowed for rapid expansion in the fields of bioinformatics and data-sharing. Resources such as the Mouse Phenome Database and PhenoGen provide researchers with assessable and reliable information, offering numerous opportunities for scientists to gain insight into potential directions of research. Continuing these trends, our group, in collaboration with Tulane Innovative Learning Center and Technology Services, has established the Zebrafish Neurophenome Project (ZNP), an online, open-source repository for behavioral and physiological data collected in the zebrafish (*Danio rerio*) model species (http://www.tulane.edu/~znpindex/). Zebrafish researchers from around the globe can input their results into ZNP, eventually generating a comprehensive resource for further scientific inquest into the zebrafish model. In the database, experiments are categorized by qualifiers such as principal investigator, zebrafish strain, experimental or pharmacological manipulation, and behavior paradigm. Key words included in the description of each experimental finding facilitate accuracy when using the ZNP search engine, which allows for advanced sorting and visualization of data. Scientists can use this database to determine effective dose ranges for pharmacological manipulations, search for specific protocols, and view the results of similar experiments. ZNP will serve a much-needed role in zebrafish research: the collection and organization of vast amounts of physiological and neurobehavioral data in a single, accessible location. The ZNP goal is to continue to provide researchers in the field of zebrafish neuroscience with novel ways in which to view large amounts of data, clarifying the scientific process and presenting new perspectives on pressing biomedical problems.

THE ASSESSMENT OF MAXIMUM PREDICTIVE VALUE IN A ZEBRAFISH ANXIOLYTIC SCREENING ASSAY. JE Warnick, A Linker, Department of Behavioral Sciences, Arkansas Tech University, Russellville, AR, USA

While predictive validity is the mainstay of assessing pharmacological screening assays, there are many limits to this evaluative standard. One of the largest deficits comes from the dichotomous nature of predictive validity. Declaring a model possesses predictive validity fails to provide any information about the level of efficacy a drug had on the primary measure or about the comparison of effects between models. These limitations led this laboratory to advocate measuring the maximum predictive value of animal models. This measure utilizes the common statistic of effect size to compare the maximum efficacy of a treatment both within and between models to allow better judgments about screening assay usage. The current study analyzed data from a zebrafish anxiolytic screening assay (Novel Tank Diving Test) to determine the maximum predictive value of multiple anxiolytics and anxiogenics. The current study used data from previous research in the laboratory of Dr. A Kalueff that examined the effect of the following drugs and chemicals in the Novel Tank Diving Test: diazepam, fluoxetine, caffeine, and alarm pheromone. The data were converted to Cohen's d scores for each drug dose. As expected, diazepam and fluoxetine showed anxiolytic effects while caffeine showed anxiogenic effects. However, the natural anxiogenic alarm pheromone was shown to produce both anxiogenic and anxiolytic results. How these data can be utilized to help define and differentiate behavioral measures of drug effects will be discussed.

12.00-13.00 Conference Mini-Symposium 5: BIOLOGICAL PSYCHIATRY II
Chairs: AV Kalueff, YV Kyrylenko (presentations 20 min)

- INTRODUCTION
- E Kyzar, S Gaikwad, M Pham, J Green, A Roth, Y Liang, V Kobla, AV Kalueff (New Orleans, USA). TOWARDS HIGH-THROUGHPUT PHENOTYPING OF COMPLEX PATTERNED BEHAVIORS IN RODENTS: FOCUS ON SELF-GROOMING
- Y Kyrylenko (Bremen, Germany) PSYCHIATRIC CASE STUDY
- L Elling, H Schupp, JBayer, A-K Bröckelmann, C Steinberg, C Dobel, M Junghofer (Muenster, Germany) ACUTE PSYCHOSOCIAL STRESS MODULATES MAGNETOENCEPHALOGRAPHIC CORRELATES OF VISUAL ATTENTION

TOWARDS HIGH-THROUGHPUT PHENOTYPING OF COMPLEX PATTERNED BEHAVIORS: LESSONS FROM MOUSE SELF-GROOMING. E Kyzar, S Gaikwad, M Pham, J Green, A Roth, Y Liang, V Kobla, AV Kalueff. Tulane University Medical School, Department of Pharmacology and Neuroscience Program, Tulane Neurophenotyping Platform, New Orleans, LA, USA

Animal self-grooming behavior is becoming increasingly recognized in neurophenotyping research. Rodent grooming and its complex sequencing are sensitive to various genetic and pharmacological manipulations. However, its phenotyping is usually limited to global endpoints such as frequency of bouts and total grooming duration. In contrast, our study focused on developing a novel, software-driven assay with the ability to quantify the complex sequencing of rodent grooming bouts. Adult male C57BL/6J mice were housed 3-5 animals per cage on a 12:12 h light:dark cycle. The animals were transferred to the experimental room 1 h before testing for acclimation. Here, we used custom-upgraded HomeCageScan video-tracking software (Clever Sys. Inc., Reston, VA), to record the grooming behavior of mice in transparent observation cylinders for 5 min. This allowed us not only to perform behavioral quantification of specific grooming patterns (such as paw licking and body/leg grooming) but also analyze the transitions within bouts, revealing significant correlations ($P<0.0005$-0.02, $R=0.51–0.70$) with manual observations for total number of transitions and selected specific grooming transitions. In addition, animals were tested for spontaneous and water-induced grooming behavior. Water-induced mice displayed more robust grooming behavior as detected by both manual observers and the custom HomeCageScan software. Both induced and novelty-evoked grooming tests show similar agreement between manual observations and software analysis, validating both models for the further study of rodent grooming behavior. This unique approach is currently being applied to the phenotyping of several mutant strains, including SERT and BDNF knockout mice. Overall, our data suggests that high-throughput automated neurophenotyping of grooming behavior can be developed for biomedical research based on this approach.

ACUTE PSYCHOSOCIAL STRESS MODULATES MAGNETOENCEPHALOGRAPHIC CORRELATES OF VISUAL ATTENTION. L Elling, H Schupp, J Bayer, A-K Bröckelmann, C Steinberg, C Dobel, M Junghofer, Institute for Biomagnetism and Biosignalanalysis, Muenster, Germany

Acute stress elicits activation of specific cerebral catecholaminergic systems. Little is known on the consequences thereof on higher cognitive functions in humans. Theoretical inferences would suggest an increased of exogenous attention, in particular towards emotionally significant stimuli. For recent suggestions on the pathogenesis of PTSD, such attentional biases are of importance. Methods: We investigated the influence of acute social stress on magnetoencephalographic (MEG) correlates of visual attention. Healthy male subjects were presented with emotional and neutral pictures in three subsequent MEG recordings while being exposed to a stressor, or to a cognitively equally demanding, but non-stressful control procedure. The stressor was deliberately designed to preserve the stress throughout the MEG recordings in order to keep the short-lived catecholaminergic stress reactions going on. Results and Discussion: Endocrine, cardiac and self-report measures of stress confirmed a notable and constant stress level over 25 minutes of MEG recordings. Concerning the attentional tuning, there was an equally constant increased N1m amplitude under acute stress, whereas the magnetic early posterior negativity (EPNm) was present, but not at all modulated by stress. This suggests that acute stress generally increases the influence of exogenous attention but does not specifically affect emotional attention. Based on the time course of the effects over the subsequent recordings, we preclude an influence of endocrine stress reactions on the MEG correlates of attention. Moreover, such a pattern may be interpreted as indicative of an influence of central noradrenaline rather than dopamine. Research Support: This research was funded by the Deutsche Forschungsgemeinschaft (DFG-FOR 751-JU445/4.2).

14.00-14.45 Plenary Lecture:
USING SCIENCE TO IMPROVE THE "SWEET SCIENCE": AN EVIDENCE-BASED APPROACH TO OFFICIATING TRAINING, ATHLETE QUALIFICATIONS AND FIGHTER SAFETY.
JE Warnick, Department of Behavioral Sciences, Arkansas Tech University, Russellville, AR, USA

While the sport of professional boxing is a widely popular billion dollar athletic enterprise, for the past century, there have been frequent calls for its ban from the medical, political and religious communities. Much of the condemnation of the sport has focused on the potential health consequences of participating in a bout (e.g., traumatic brain injury), the integrity of the ring officials, and the ability for unqualified athletes (e.g., the aged, those with health concerns, etc.) to participate. What if the boxing community used evidence-based practices to reform the sport? This laboratory has found methods to address each of these issues by relying on scientific findings. This lecture will highlight previous evidence-based practices that have improved the sport of boxing and will provide potential solutions to the problems that threaten to cause its demise.

14.45-16.00 Conference Symposium 6: ARKANSAS TECH UNIVERSITY-TULANE COLLABORATIVE INITIATIVE SYMPOSIUM ON STRESS PSYCHOLOGY
Chair: JE Warnick (presentations 20 min)

- INTRODUCTION
- D Hughes, WD Martin, J Warnick (Russellville, USA) LINGUISTIC ANALYSIS OF PRECOMPETITION INTERVIEWS PREDICT THE OUTCOME OF PROFESSIONAL BOXING BOUTS
- D Hughes, B Mader, A Linker, J Osben, R Williams, WD Martin, JE Warnick (Russellville, USA) THE INFLUENCE OF THE "HOME FIELD ADVANTAGE" IN PROFESSIONAL BOXING
- B Mader, J Osben, D Hughes, A Linker, WD Martin, JE Warnick (Russellville, USA) INVESTIGATING THE ABILITY OF THE STRUGGLE INDEX SCORE TO QUANTITATIVELY SUMMARIZE A PROFESSIONAL BOXING BOUT
- DISCUSSION

LINGUISTIC ANALYSIS OF PRECOMPETITION INTERVIEWS PREDICT THE OUTCOME OF PROFESSIONAL BOXING BOUTS. D Hughes, WD Martin, JE Warnick. Department of Behavioral Sciences, Arkansas Tech University, Russellville, AR, USA

In spite of a rich history, combative sports have received little attention from the behavioral sciences aside a focus on traumatic brain injury. This experiment attempted to further determine which psychological variables are correlated with the outcome of a professional boxing bout. Methods: Interviews, press conference quotes, and press release statements given by professional boxers prior to a boxing match over a 21 month time-span were collected from a major combative sports news website (www.fightnews.com). The textual data for each fighter were analyzed by the linguistic software LIWC2007 (Linguistic Inquiry and Word Count). The outcome of each fighter's bout(s) was retrieved from an online database of professional boxing results (www.boxrec.com). Results and Discussion: Correlation analysis revealed that the pre-bout use of words conveying positive emotions, focusing on health and work were related to winning a bout. Increased grammatical nuances (function words, pronouns, third person plural words, impersonal pronouns, auxiliary verbs, and past tense) and the use of words focusing on social functions and conveying tentativeness before a bout were related to losing the athletic contest. This investigation provides a starting point for future studies using direct measures on combative sport participants. These findings suggest measures of mood, health, motivation, self-efficacy, social relationships, and identity could be related to performance differences in professional boxing. The current study will be discussed in the context of previous research from this laboratory.

THE INFLUENCE OF THE "HOME FIELD ADVANTAGE" IN PROFESSIONAL BOXING. D Hughes, B Mader, A Linker, J Osben, R Williams, WD Martin, JE Warnick. Department of Behavioral Sciences, Arkansas Tech University, Russellville, AR, USA

The home field advantage effect has been a topic of frequent investigation in sport psychology. Competition occurring at a venue in a team or competitor's home town or home country has been shown to be related to success and in some circumstances, like a championship event, failure. Boxing is a fairly mobile sport where athletes typically compete in bouts occurring in a variety of venues, cities, and countries. Previous research in this laboratory has shown a home-country effect in professional boxing championship bouts, but a better characterization of the home field advantage effect in this sport is needed. Methods: The career win-loss rates for individual boxers at specific venues were obtained from an online boxing database (www.boxrec.com). Boxer win-loss rates for venues (N = 4,127) was narrowed down to cases with multiple

bouts occurring at a single venue (N = 812). Results and Discussion: Correlation analysis revealed that the multiple bouts at a common venue was related to winning a bout. Further analyses investigating home-city and home-country effects will be presented. Similar to other sports (e.g., baseball, basketball, amateur wrestling), professional boxing bouts are subject to a home-field advantage effect. Future research utilizing direct measures of contestants to determine the psychological underpinning of this effect are currently being planned by this laboratory.

INVESTIGATING THE ABILITY OF THE STRUGGLE INDEX SCORE TO QUANTITATIVELY SUMMARIZE A PROFESSIONAL BOXING BOUT. B Mader, J Osben, D Hughes, A Linker, WD Martin, JE Warnick. Department of Behavioral Sciences, Arkansas Tech University, Russellville, AR, USA

Recently, a method of analyzing combative sports was introduced that reduced the participants' actions into a quantitative score called the struggle index score. This method has been used to investigate fencing, taekwondo, judo, and amateur boxing and is able to differentiate superior and inferior talent, distinguish the actions most likely to predict victory, and, some have argued that, it can differentiate bouts that are aesthetically pleasing for the audience (e.g., action filled contests). The current study applies this quantitative method to the sport of professional boxing to determine whether the struggle index score is capable of capturing the dynamics of this sport. Methods: Raters were asked to score five rounds of boxing (inter-rater reliability ≥ .98) which were judged by Ring Magazine to be the round of the year in 2005, 2006, 2007, and 2008. The fifth round scored is widely considered to be one of the most one-sided rounds in modern history (Round 6 of Mayweather-Gatti, 2005). The scorers divided each round into multiple 10-second segments and manually scored the following actions: 1) punches thrown, 2) punches landed, 3) counterattack thrown, 4) counterattack landed, and 5) defensive actions. This data was used to calculate the struggle index score. Results and Discussion: The results demonstrate that the struggle index score fails to accurately describe a boxing bout. Unlike for other combative sports, the losing contestants in professional boxing bouts were found to have a larger struggle index score. The more accurate reflection of successful boxing combatant was found to be a simple percentage of successful offensive action. However, this score did not appear to provide any insight into aesthetic quality of the bout as the mismatch had higher offensive action scores than the round of the year. The reasons for this disparity between boxing and other combative sports will be discussed.

16.30-17.40 GUIDED POSTER SESSION

- Y Shirakawa, A Senoo, S Nakamura, M Koshiba (Tokyo, Japan) CORRELATION ANALYSIS OF BRAIN MONOAMINES AND SOCIO-EMOTIONAL BEHAVIORS
- A Stewart, S Gaikwad, AV Kalueff (New Orleans, USA) SPATIOTEMPORAL STABILITY OF ADULT ZEBRAFISH EXPLORATION IN A NOVEL ENVIRONMENT
- S Gaikwad, E Kyzar, A Stewart, A Roth, J Green, M Pham, AV Kalueff (New Orleans, USA) VISUALIZING ADULT ZEBRAFISH 3D BEHAVIOR AND ITS UTILITY FOR NEUROSCIENCE RESEARCH
- J Green, S Gaikwad, E Kyzar, A Stewart, A Roth, M Pham, AV Kalueff (New Orleans, USA). ANXIOGENIC EFFECTS OF AMPHETAMINE IN ZEBRAFISH
- M Pham, P Hart, E Kyzar, S Gaikwad, J Green, A Roth, R Riehl, M Pham, AV Kalueff. MODELING THE EFFECTS OF KETAMINE IN ZEBRAFISH (DANIO RERIO)
- S Chanin, C Fryar, D Varga, J Raymond, E Kyzar, J Enriquez, S Bagawandoss, S Gaikwad, A Roth, M Pham, I Zapolsky, I Bruce, J Hester, J Green, D Desmond, A Stewart, AV Kalueff (New Orleans, USA). ASSESSING STARTLE RESPONSES AND THEIR HABITUATION IN ADULT ZEBRAFISH
- K Mimura, S Nakamura, M Koshiba (Tokyo, Japan) MULTIVARIATE REPRESENTAITON OF CHICK'S SOCIAL BEHAVIORAL DEVELOPMENT AND CORRELATED CELL STRUCTURAL MODULATION IN AMYGDALA CORE NUCLEUS

CORRELATION ANALYSIS OF BRAIN MONOAMINES AND SOCIO-EMOTIONAL BEHABIORS. Y Shirakawa, A Senoo, S Nakamura, M Koshiba, Tokyo University of Agriculture and Technology, Tokyo, Japan

Monoamine plays an important role in the assessment of sensory information and the motivation for action. We established the socio-emotional development model in domestic chicks (Gallus gallus domesicus), which were raised under social interaction rich (grouped; Grp) or poor (isolated; Iso) conditions during a high sensitive period for acquisition of peer social affiliation behavior. Grp was likely to gather with other peers, in contrast, Iso was prone to escape/freeze in the face of others. To explore the neuronal mechanism, we

compared the brain monoamine levels between Grp and Iso just after .a 20-minute social test. Methods: White leghorn (Maria) chick was reared as grouped or socially-isolated conditions from just after hatching. Female chick was left alone or kept in a group for 20 min under video-recordings, immediately followed by brain monoamine preparation from 13 brain regions. Monoamines including dopamine (DA), serotonin (5-HT), norepinephrine (NE) and their metabolites were analyzed by HPLC-ECD. The relative value of each monoamine to total monoamines was used to cancel sampling error. Results and Discussion: Homovanillic acid (metabolite of DA; HVA) decreased and 5-HT increased in the medio-frontal association area of forebrain under short-term isolation in both Grp and Iso. Furthermore, it was suggested that HVA increased and 5-HT decreased in the same area due to long-term isolation. These consistent results with the antagonistic interaction between DA and 5-HT may imply the developmental mechanism of socio-emotional network. Research Support: MEXT Grant-in-Aid for Scientific Research on Innovative Areas 21200017.

SPATIOTEMPORAL STABILITY OF ADULT ZEBRAFISH EXPLORATION IN A NOVEL ENVIRONMENT. A Stewart, S Gaikwad, AV Kalueff, Department of Pharmacology and Neuroscience Program, Tulane Neurophenotyping Platform, Tulane University Medical School, 1430 Tulane Ave., New Orleans, LA, USA

Zebrafish (Danio rerio) have emerged as a useful model organism in neurobehavioral research. Mounting evidence suggests that similar strategies of novelty exploration are used between rodents and zebrafish. However, while numerous rodent paradigms have been adapted for testing zebrafish behavior, the exact nature of zebrafish exploration patterning in a novel environment is not fully understood. Methods: The open field test is a popular rodent test recently applied to zebrafish research. To better understand their exploration, we exposed adult zebrafish to different open field arenas of varying size for 6 and 30 min. The amount and temporal patterning of their exploration, assessed as a per-minute distribution of activity, was then analyzed for several key behavioral endpoints. Results and Discussion: We showed that zebrafish adjust their exploratory locomotion to the size of the novel arena, while the temporal patterning of their activity remains unaltered. The total distance traveled differed significantly between arenas, while the total velocity, immobility duration, immobility frequency, inter-stop distance, and average immobility remained consistent throughout the differing arenas for both the 6 and 30 min. trials. Examining a per-minute distribution of their horizontal activity, we found that temporal patterning in a given minute of the trial remained stable regardless of the arena used. Interestingly, we also observed a somewhat sigmoidal temporal patterning in activity, with the per-minute behavioral distribution exhibiting \"waves\" of exploration across the trials. This spatiotemporal “stability” is also in line with previous rodent studies, suggesting that the spatiotemporal patterning of zebrafish behavior is conserved and unaffected by external influences. Research Support: The study was supported by Tulane University Intramural funds, Zebrafish National Research Consortium (ZNRC), Provost’s Scholarly Enrichment Fund, Newcomb Fellows Grant, and LA Board of Regents Pfund grant.

VISUALIZING ADULT ZEBRAFISH 3D BEHAVIOR AND ITS UTILITY FOR NEUROSCIENCE RESEARCH. S Gaikwad, A Stewart, A Roth, J Green, E Kyzar, M Pham, AV Kalueff. Tulane University Medical School, Department of Pharmacology and Neuroscience Program, Tulane Neurophenotyping Platform, New Orleans, LA, USA

The use of adult zebrafish (Danio rerio) in neurobehavioral research is rapidly developing. The aim of the present large-scale study was to characterize complex behavioral responses to various stressors and to investigate the potential in new computer-generated endpoints, in order to construct a more precise dissection of zebrafish behavior. To better understand zebrafish behavioral phenotypes, we generated temporal and spatial three-dimensional (3D) reconstructions of zebrafish swimming activity in the novel tank test, globally assessed multiple anxiogenic and anxiolytic manipulations, examined all behavioral endpoints within 3D reconstructions, and performed cluster analysis to identify behavioral markers of high and low anxiety states in zebrafish. A combination of these approaches (Cachat et al. 2011) provides an innovative approach to high-throughput behavioral phenotyping of adult zebrafish, aiming to improve our understanding of their stress-related behavior.

ANXIOGENIC EFFECTS OF AMPHETAMINE IN ZEBRAFISH. J Green, S Gaikwad, E Kyzar, A Stewart, A Roth, M Pham, AV Kalueff. Tulane University Medical School, Department of Pharmacology and Neuroscience Program, New Orleans, LA, USA

Adult zebrafish have become a widely used animal model in neurobehavioral research, as their rich behavioral repertoire allows for the characterization of complex behavioral phenotypes. In our current study, we have used new techniques to visualize and quantify amphetamine evoked anxiety-related behavior in zebrafish. The novel tank test is a paradigm that utilizes the innate diving response and aversion to novel environments to assess stress responses in fish. Initially, the fish will bottom-dwell; however, as the fish

acclimates to the environment, it gradually explores the upper portions of the tank. When exposed to amphetamine, zebrafish exhibit markedly reduced transitions to and time spent in the upper half of the tank. This anxiety-like response is dose-dependently modulated, as higher doses increase avoidance behavior and decrease exploration. Interestingly, challenge from 10 mg/L of amphetamine evoked a marked increase in c-fos expression in the brain, suggesting the wide spread activatory/pro-arousal effects of amphetamine on neural pathways. Interestingly, these effects of amphetamine resemble those of another similar agent, cocaine, in zebrafish (Stewart et al., 2011). Overall, our results suggest that adult zebrafish can be useful in identifying and characterizing behavioral phenotypes precipitated by pharmacological manipulation, including anxiogenic effects of selected drugs of abuse, such as amphetamine. Furthermore, this model may also be useful in screening the effects of novel compounds on zebrafish anxiety related behavior.

ASSESSING ANXIOLYTIC–LIKE EFFECTS OF KETAMINE IN ZEBRAFISH (DANIO RERIO). M Pham, E Kyzar, S Gaikwad, J Green, A Roth, M Pham, AV Kalueff. Tulane University Medical School, Department of Pharmacology and Neuroscience Program, Tulane Neurophenotyping Platform, New Orleans, LA, USA

Zebrafish (Danio rerio) have recently become a common animal model for biomedical and neuroscience research. Here, we applied several testing paradigms to characterize the effects of exposure to acute ketamine (a glutamatergic NMDA antagonist) in adult zebrafish. The novel tank diving paradigm takes advantage of the innate bottom-dwelling response of zebrafish placed in novel environments, where diving and reduced top exploration indicate anxiety. The experimental subject will gradually begin to explore the upper portion of the tank as it habituates. Ketamine (20 and 40 mg/L, by immersion) elicited increased duration of time spent in the upper half of the tank, decreased latency of initial top entry, and decreased freezing behavior. Increased circling behavior has also been observed in ketamine-treated fish (strikingly paralleling ketamine-evoked circling in rodents). Zebrafish behavior was also analyzed through video-tracking (Noldus EthoVision XT7), which revealed reduced velocity and meandering for ketamine-treated fish. In the shoaling test paradigm, average inter-fish distance is used as a reliable marker of anxiety, as fish tend to remain in close proximity. Ketamine produced longer inter-fish distances in a dose-dependent manner. Fish treated with ketamine also showed increased time and transitions in the illuminated portion of the light-dark box, once again supporting our findings of ketamine as an anxiolytic. Lastly, ketamine produced circling behavior in the open field test, which markedly resembles ketamine-evoked rotational behavior in rodents. The drug also produces decreases whole-body cortisol in zebrafish, which once again supports ketamine's anxiolytic profile, collectively confirming the behavioral sensitivity of adult zebrafish models to anxiolytic/hallucinogenic glutamatergic drugs.

ASSESSING STARTLE RESPONSES AND THEIR HABITUATION IN ADULT ZEBRAFISH. S Chanin, C Fryar, D Varga, J Raymond, E Kyzar, J Enriquez, S Bagawandoss, S Gaikwad, A Roth, M Pham, I Zapolsky, I Bruce, J Hester, J Green, D Desmond, A Stewart, AV Kalueff. Tulane University Medical School, Department of Pharmacology and Neuroscience Program, Tulane Neurophenotyping Platform, New Orleans, LA, USA

Zebrafish is a popular model species for neurobehavioral and psychopharmacological study focusing on anxiety-related behaviors. This model organism has high homology to humans, possessing all classical neurotransmitters and brain structures. The startle response is an organism's instinctive, evolutionarily conserved reaction to an aversive stimulus. While startle is a well-established assay for anxiety-like behaviors in multiple species, little research has been conducted on the startle response or its habituation in adult zebrafish. Examining the startle response and its habituation is important for assessing anxiety-related behaviors, and may have high translational value for higher organisms. In the present study, adult zebrafish were individually placed in a novel tank, allowed to acclimate to the arena, and then video-recorded for 10 minutes while receiving a startle-evoking stimulus once every minute. The recorded videos were then analyzed using the Ethovision software (Noldus IT, Wageningen, The Netherlands) for endpoints which proved sensitive to startle-related behaviors such as average velocity and distance traveled. The results demonstrate a robust phenotype for startle response behavior and show clear patterns of habituation to the stimulus. Testing startle reflex behavior in zebrafish is inexpensive, effective, and easily reproducible, and the method outlined here allows for simultaneous testing of the startle response and the organism's habituation to the stimulus within a single experiment. In addition to drug-free studies, startle response and its habituation have multiple applications in pharmacological screening and phenotyping of mutant and transgenic zebrafish strains.

MULTIVARIATE REPRESENTAITON OF CHICK'S SOCIAL BEHAVIORAL DEVELOPMENT AND CORRELATED CELL STRUCTURAL MODULATION IN AMYGDALE CORE NUCLEUS. K Mimura, S Nakamura, M Koshiba, Tokyo University of Agriculture and Technology, Tokyo, Japan

How social emotion and recognition develops in children is the central issue in cognitive neuroscience and biological psychiatry. To comprehend the basis of social disorders like autism as the output of the neural network, we developed a new animal model of social development represented by quantitative multivariate analysis. Methods: We reared domestic chick (gallus gallus domesticus) under the grouped with peers (Grp) or socially isolated (Iso) conditions and traced the developmental process of peer-socializing behaviors in face-to-face scene from postnatal day 3 to 14 (P3-14). The principal component analysis and clustering analysis was performed by the parameters measured from the video-recorded behavioral data and the neural cells in amygdale complex stained by Nissl and Hoechst. Results and Discussion: We found the developmental process significantly different between Grp and Iso chicks. The parameters contributed to the difference were not only in locomotive activity but also in head rotation velocity and frequency of turning head to peers. The Nissl-positive cell number in Grp chicks was significantly higher in amygdale core nucleus (ACo). Furthermore, the cluster analysis suggested that the cell number correlated with the subject's local preference where it was closer to peers in the test cage and joyful call frequency. The neural marker expressed in the nissl-positive cells in ACo remains to be clarified, which may tell us a neuronal mechanism of social affiliation behavior. Research Support: MEXT Grant-in-Aid for Scientific Research on Innovative Areas 21200017

17.40-18.00 Closing Ceremony.
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