Abstract 1

IMPAIRED KIR4.1 CHANNEL FUNCTION DUE TO KCNJ10 VARIATIONS

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KCNJ10-gene mutations are causal in certain forms of epilepsy. Kir4.1 channels encoded by KCNJ10 generate the major K+ conductance in astrocytes and are essential for buffering extracellular [K+]. The KCNJ10 gene contains over 120 coding-region single nucleotide polymorphisms (SNPs). We hypothesize that uncharacterized Q212R, L166Q and G83V SNPs alter Kir4.1 channel function.

The impact of these SNPs on homomeric Kir4.1 and heteromeric Kir4.1/5.1 channel function was evaluated using whole-cell and inside-out patch clamp from tSA201 cells expressing WT Kir4.1 or its variants. We analyzed: i) membrane potential, ii) whole-cell currents, iii) barium block of voltage induced currents, iv) response to increased extracellular [K+] and v) pH dependence.

There was no difference between the Q212R variant and the WT Kir4.1 channel. The G83V variant did not display channel function under any conditions tested. The homomeric L166Q channels had reduced whole-cell current, barium block and response to elevated extracellular K+. Unexpectedly, function was rescued upon co-expression with the Kir5.1 subunit.

Our data suggest that SNPs of KCNJ10 may affect channel function via different mechanisms that could lead to hyperexcitability and neuronal death.

Abstract 2

SEX-SPECIFIC EXPRESSION OF MU OPIOID RECEPTOR IN BRAIN STRUCTURES INVOLVED IN FEAR CONDITIONING AND EXTINCTION LEARNING

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Ponce School of Medicine and Health Sciences

Intercommunication between the amygdala medial prefrontal cortex mPFC and the periaqueductal gray PAG mediates certain aspects of fear extinction learning Studies using cued fear-conditioning showed that acute morphine facilitates extinction in male rats In contrast we have shown that fear conditioned female rats given morphine during the metaestrus (M low hormones stage of the estrous cycle showed increased recall of fear expression Female rats that received morphine during the proestrus (P high hormones stage and male rats showed normal conditioning and extinction learning To further assess the sex-specific effects produced by morphine we examined mu opioid receptor (MOR expression in several brain regions Male and female P and M rats underwent auditory fear-conditioning followed by a morphin e 2.5 mg/kg s.c or saline injection The next day rats underwent extinction training and were sacrificed Western blots were used to measure MOR in PAG amygdala and mPFC as a percent of change from controls. In M females low MOR expression in the amygdala was associated with increased fear responses during conditioning recall. Morphine given after traumatic events to women with low-ovarian hormones might alter the recall of fear-responses.

Abstract 3

THE EFFECTS OF CAFFEINE ON THE INTRINSIC PROPERTIES OF VENTRAL HORN NEURONS

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Institute of Neurobiology1
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Caffeine is the most consumed psychoactive substance worldwide with significant effects on the activity of neuronal pathways in the central nervous system. Caffeine increase locomotor activity in rodents and this has been linked to the inhibition of A1 and A2a adenosine receptors, with a major role in regulating pain and the inflammatory response in patients after suffering a spinal cord injury (SCI).The effects of caffeine at the level of a single network such as the spinal central pattern generator (CPG) network for hindlimb locomotion are lacking. The purpose of this study is to elucidate the effects of caffeine modulation on adenosine receptors of spinal neurons. Direct caffeine application (50µM) to spinal cord slices, affects the membrane properties of spinal interneurons by decreasing the threshold and after-hyperpolarization (AHP) of the action potential, resulting in excitation of these neurons. Our results suggest that caffeine modulates the intrinsic properties of the component neurons of the spinal network controlling locomotion.

Abstract 4

AN AVOIDANCE-BASED MODEL OF EXPOSURE WITH RESPONSE PREVENTION IN RATS

José Rodríguez-Romaguera[1], Héctor Bravo-Rivera[1], Estefanía I. González-Araya[1], Angélica M. Minier-Toribo[1], Benjamin D. Greenberg[2], Steve A. Rasmussen[2], and Gregory J. Quirk[1]

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Patients suffering from harm-avoidant type of obsessive-compulsive disorder (OCD) believe their compulsions protect them from danger (Rasmussen, 1992). Compulsions can be viewed as persistent avoidance responses. OCD is treated with exposure-with-response-prevention (ERP) therapy, where patients are repeatedly exposed to trigger stimuli but prevented from expressing compulsions. ERP extinguishes avoidance responses. We therefore developed an extinction-with-response-prevention ("Ext-RP") task in rats. Initially rats learn to avoid a tone-signaled shock by stepping onto a platform (Bravo-Rivera et al., 2014). Following avoidance training, access to the platform was blocked with a Plexiglas barrier during 3 days of extinction (resembling ERP). The following day, the barrier was removed to test for avoidance and 25% of rats persistent to avoid. DBS of the ventral capsule/ventral striatum (VC/VS) improves ERP response in OCD patients (Greenberg et al., 2006; Denys et al., 2010). We therefore applied DBS to VS in persistent avoidance rats, during an additional Ext-RP session. DBS did not reduce freezing during Ext-RP, but abolished persistent avoidance the following day.

Abstract 5

LOCOMOTOR ANALYSIS OF *DROSOPHILA* MUTANTS AFFECTING NEUROTRANSMITTER RELEASE.

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Introduction: Neurotransmitter-release is regulated by several presynaptic-proteins where Syntaxin and Complexin are crucial. We look the locomotor activity of *Drosophila* adult animals to evaluate if altered motor behavior could be a consequence of an abnormal central nervous system or altered peripheral nervous system output.
Hypothesis: Mutants display poorer motor performance in agreement with altered synaptic transmission.
Methods: To discriminate between central and peripheral motor alteration we look at the locomotor behavior in animals with and without head. Climbing assays, grooming and flip over were tested in entire animals. Stepping and flip over were tested in decapitated individuals.
Results: Complexin null and Syntaxin hypomorph mutant display similar phenotype in synaptic transmission but different behaviors. Complexin could not climb, Syntaxin has the lower climbing speed and the other genotypes have indistinguishable performance. Complexin flip over in 15min in turn, other genotypes flip over in few seconds. Decapitated animals are not able to climb but they stay on their feet readjusting the position. Surprisingly, decapitated animals are able to fly without control and flip over. Complexin and Syntaxin display the slower performance compared with the other genotypes.
Conclusion: Locomotion is a complex behavior which includes central and peripheral control. Flight and stepping is encoded in an independent circuit in the peripheral nervous system in turn navigation requires the central nervous system. Severe synaptic transmission defects observed in Complexin impair all type of locomotion. Less severe synaptic transmission defects allow the animals to perform all types of locomotion but with lower performance.

Abstract 6

DEPRESSION IN VASCULAR DEMENTIA

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Fundación Blue Science, Dominican Republic

Objectives:
Determine the level of depression in vascular dementia patients.

Methods:
A clinical, descriptive, controlled, randomized prospective study was performed on a sample of 40 patients of both sexes from ages 60 to 80 years of the Outpatient Psychiatry Department of the Hospital Plaza de la Salud in Santo Domingo, DominicanRepublic with the diagnosis of dementia, where patients suffering from vascular dementia and the level of depression was measured with the Hamilton Depression Rating Scale in the time of three months were chosen. Vascular Dementia diagnosis was determined by MRI and indicated laboratory tests of lipids and glucose.

Results:
The sample showed that 53 % were female, as described by the statistics. Depression 100%, 76% hypertension, 62% presented with total cholesterol and LDL, with positive symptoms of mental examination: 100 % depression, 94% insomnia, 94% hypomnesia, moderate depression 53 %.

Conclusions:
We conclude the sample were mostly female, presenting increased total and LDL cholesterol and all depressives with more than half had moderate depression. Patients with VD in this sample had depression, mostly moderate depression.. Must be considered by the clinician discard depression in patients with vascular dementia.

Abstract 7

How TBI affects wild-type mice in comparison with transgenic AD models

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Traumatic brain injury TBI results from a force transmitted to the head that leads to a collision between the brain and skull that may result in impairment of normal brain function. We hypothesize that TBI and Alzheimer's disease AD share common pathways that lead to neuronal dysfunction and the goal of this study is to characterize the convergence of TBI and AD at the cellular level We compared the cellular changes induced by TBI in mice that received a pressurized air blast to the skull to naieve controls We have two important sources of data to evaluate the effects of TBI in our mice memory function and the quantification of AD biomarkers such as A and tau The behavioral analysis of fear conditioning done following TBI and in sham control mice suggests that the control animals retained the memory of the context in which they were shocked better and for longer than the blasted animals Immunohistochemistry for A and P-tau did not reveal a detectable increase in these AD biomarkers Our behavioral data suggests that TBI alters hippocampal function at 1 month post-blast but does not induce hallmarks of neurodegeneration

Abstract 8

ELUCIDATING THE EVOLUTIONARY HISTORY OF HOMOPHILIC BINDING MOLECULES IN NEURAL NETWORK FORMATION USING THE BASAL CHORDATE CIONA INTESTINALIS

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During nervous system development neurons recognize self from non self while extending axons and dendrites. This is mediated by a complex array of molecules that provides identity to each neuron. In insects, the Ig cell adhesion molecule DSCAM confers specificity with 18,000 potential isoforms expressed stochastically in each neuron. In vertebrates, g-protocadherins display similar isoform diversity but use a different mechanism for cellular identity. In both instances homophilic cell adhesion is the key molecular process that mediates cellular recognition. To gain insight into the evolutionary pressures of homophilic binding in neural network formation, we used the marine tunicate Ciona intestinalis. Tunicates are basal chordates with a simplified cellular composition, a compacted genome and a vertebrate-like body plan. We have found 3 members of the IgCAM family in Ciona but neither displays genetic diversity. Given that Ciona has simplified neuronal and neural network morphology, we hypothesize that isoform diversity led to the complexities of neural networks whereas the lack of diversity results in simpler neuronal morphology.

Abstract 9

NEURONAL ACTIVITY IN PRELIMBIC CORTEX CORRELATES WITH EXPRESSION OF ACTIVE AVOIDANCE

Bravo-Rivera, Christian, Diehl, María M., Roman-Ortiz, Ciorana, Pagan-Rivera, Pablo A., and Quirk, Gregory J.

University of Puerto Rico, Medical Sciences Campus

We recently reported that the prelimbic prefrontal cortex (PL) is necessary for platform-mediated avoidance, a novel avoidance task in which rats avoid a tone-signaled foot shock by stepping onto a platform. In the current study, we recorded single-unit to determine if PL neurons are responsive to the tone, platform-approach, or both, in rats performing avoidance. We found that 23% of PL neurons were tone responsive cells (TR0cells), which is comparable to fear conditioning (25%, Burgos-Robles et al., 2009). Interestingly, avoidance was associated with a higher percentage of inhibitory TR-cells than fear conditioning (19% vs. 5%). We next examined neural correlates of platform approach. 38% of PL neurons signaled platform-approach, which was significantly higher than in an unconditioned group (13%, $p<0.05$), suggesting that PL activity reflects avoidance behavior. Platform-approach responses were more often excitatory than inhibitory (39% vs. 16%, $p<0.01$). Most cells that responded to the tone also responded to approach (63%), and vice-versa (61%). Together, our findings suggest that inhibitory tone responses in PL emerge with avoidance and that inputs signaling the tone converge with inputs signaling platform approach.

Abstract 10

AVOIDANCE PERSISTENCE CORRELATES WITH PREFRONTAL AND STRIATAL ACTIVITY

Ciorana Roman-Ortiz, Christian Bravo-Rivera, Marlian Montesino-Cartagena, and Gregory J. Quirk

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Avoidance is a symptom of anxiety disorders, and is often resistant to extinction. To identify avoidance structures, we developed a task in which rats avoid a signaled footshock by stepping onto a safe platform. We reported that the prelimbic cortex (PL), ventral striatum (VS) and basal amygdala (BA) are necessary for avoidance expression, whereas the infralimbic cortex (IL) is necessary for avoidance extinction. Here, we aimed to identify structures involved in extinction-resistant avoidance. We measured cFos in different structures to map regions active in avoidance extinction. We found that activity in PL, VS, and BA positively correlated with avoidance, whereas activity in IL correlated negatively with avoidance. Extinction failure likely occurred because of decreased IL activity, which would normally decrease fear by inhibiting BA. Does avoidance occur despite successful fear extinction? To test this, we presented unreinforced tones over days without the platform until rats expressed fear extinction retention. We also observed that activity in PL and VS correlated with avoidance; however, IL and BA activity showed no correlation. These findings suggest that persistence of avoidance can occur with or without deficits in extinction.

Abstract 11

DEVELOPMENT OF INTRASPINAL SEROTONERGIC NEURONS IN ZEBRAFISH

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Intraspinal Serotonergic Neurons (ISNs) are serotonin-producing cells in spinal cords of zebrafish. Previous data showed that the number and morphology of ISNs varied during development, and the neurons at 48hpf were different from cells at 72hpf. This data suggests that cells at these stages represent different neuron populations. To confirm these findings, we used different markers to study ISN development in zebrafish. A transgenic line expressing fluorescence in the pet1 transcription factor and tph2 gene (both important in ISN development) was used to identify ISNs through development. Another line expressing fluorescence in Kolmer-Agdhur cells was used to mark their development. At early stages, serotonin-positive neurons were negative for pet1 and tph2. Later in development, those cells displayed pet1 and tph2 expression, suggesting that early cells may not be ISNs, and a population of ISNs appear at later stages. In the Kolmer-Agdhur line, some cells were serotonin- positive, meaning Kolmer-Agdhur cells may be early ISNs. This phenomenon requires further study to understand the role of ISNs in zebrafish.

Abstract 12

BIDIRECTIONAL REGULATION OF SYNCHRONOUS, ASYNCHRONOUS AND DELAY RELEASE SHORT-TERM PLASTICITIES BY COMPLEXIN.

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Synaptic vesicle fusion and cycling are crucial for neurotransmitter release and plasticity. Complexin clamps the spontaneous fusion, promotes nerve-evoked release and has been involved in the availability of vesicle for fusion. However, the role Complexin in synaptic function is still under investigation. We investigate the effects of Complexin in the releasable pools and cycling at Drosophila. Complexin enhances the Synchronous/Asynchronous release and reduces the strontium sensitivity for fusion. Delayed asynchronous release induced by high-frequency stimulation is suppressed by Complexin and EGTA-AM. Desensitization experiments discard the post synaptic receptor saturation during delayed release. Complexin null display longer delayed release kinetics, activated at lower frequencies and accelerated at 100Hz in Complexin over expression. This results indicates that Complexin suppress the asynchronous release activation either by strontium or by tetanic stimulation. Deconvolution analysis indicates that the synchronous, asynchronous and delayed releases are bidirectionally regulated by complexin and suggest different rates of replenishments.

Abstract 13

PHARMACOLOGICAL INHIBITION OF PYK2 REDUCES PRO-MIGRATORY EFFECT OF MICROGLIA ON GLIOMA TUMOR.

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Microglia infiltrate most gliomas promoting tumor dispersal. Using cell biology approaches we demonstrated that microglia stimulate glioma cell invasion through a proline rich tyrosine kinase 2 (Pyk2) signaling pathway in glioma cells. This effect is significantly reduced in presence of Pyk2 inhibitor, PF-562271. We hypothesized that supplementing current therapeutic drugs with a PF-562271 can reduce tumor dispersal and increase the effectiveness of treatment. Combined treatment of temozolomide (TMZ) and PF-562271 as compared to the current treatment of TMZ alone was investigated in vitro and in vivo. Human glioma cell lines were used for in vitro migration assays. In vivo tumor size and invasion area was determined using Hematoxylin and Eosin staining. Our data suggest that PF-562271 and TMZ eliminate microglial effect on migration of glioma cells. Furthermore, in vivo experiments demonstrated that PF-562271 reduced invasion of glioma cells at the tumor edge while TMZ reduced tumor growth.

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Abstract 14

MODELING THE DROSOPHILA MELANOGASTER GIANT FIBER (GF) NEURON

Sami H. Jezzini, Adeline Pezier, and Jonathan Blagburn.

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The bilaterally paired GF neurons are super-fast sensory integrators that help to orchestrate the escape response. Because the circuit is optimized for speed it relies heavily on the fastest form of synaptic transmission -- the electrical gap junction. We have previously found that the synapse appears to exhibit activity dependent synaptic depression which is unexpected for an electrical connection. Furthermore, increasing the number of gap junctions at this synapse, via expression of the transcription factor Engrailed, can alter this plasticity.

To further understand these observations, we are combining data from electrophysiology and confocal microscopy to develop a morphologically realistic computer model of the GF. The model will be used to investigate how the cell's geometry can affect its integrative properties. The ultimate goal of the model is to test how intrinsic properties of the GF neuron might contribute to the fast integration of electrical sensory input. This work uses openly available computational resources including the NEURON simulator and Scientific Python analysis tools.

This work is supported by NINDS SC1NSS081726.

Abstract 15

ASTROCYTES IN HYPERGLYCEMIC CONDITIONS HAVE DECREASED EXPRESSION OF TREK-2 AND KIR4.1 POTASSIUM CHANNELS.

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Type I and type II diabetes affect the central nervous system (CNS) and increase the incidence of seizure and stroke. Astrocytes contain potassium channels that are critical for normal CNS function. Loss or dysfunction of astrocytic potassium channels increases seizure susceptibility associated with ischemic brain damage. We hypothesize that high glucose alters the expression of Kir4.1 and TREK-2 potassium channels in astrocytes. To test this, primary rat astrocyte cultures were prepared and maintained in normal and high glucose medium. To assess gene and protein levels, RT-PCR and Western Blot were performed and results demonstrated decreased mRNA and proteins levels for both channels in high glucose. Whole cell patch clamp recording revealed K+ channel dysfunction in high glucose. Furthermore, glutamate uptake capabilities were impaired in high glucose astrocytes. Time course experiments showed decreased Kir4.1 protein levels and recovery at day 7. Our results suggest that decreased astrocytic potassium channels by elevated glucose may contribute to the underlying pathophysiology of diabetes-induced CNS disorders.

Abstract 16

ACQUISITION OF SPONTANEOUS MORPHINE-WITHDRAWAL CONDITIONED PLACE AVERSION IN MALE AND FEMALE RATS

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Opiate withdrawal generates negative affective states, which play an important role in craving, and compulsive drug use. In animal research, morphine withdrawal-induced conditioned place aversion (CPA) has been widely used to investigate the negative consequences of drug withdrawal mostly in males. Our objective was to test the working hypothesis that male and cycling female rats will differ in the contextual associations generated during spontaneous withdrawal from chronic morphine. Adult rats were exposed to chronic morphine injections and somatic signs of spontaneous morphine withdrawal were assessed at 14 and 84 hours after the last injection. At 14 hours after the last morphine injection (early withdrawal), we confined them to one side of a dual choice box. Our preliminary data shows a significant avoidance of the withdrawal-paired context in morphine treated females but not in morphine treated males and ovariectomized females. This suggests a sex-specific effect morphine withdrawal in contextual learning potentially affecting other drug-associated behaviors.

Abstract 17

PROBABILITY OF SPONTANEOUS TRANSMITTER RELEASE AT INDIVIDUAL ACTIVE ZONES.

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Synaptic transmission is mediated via vesicle fusion and neurotransmitter release. Vesicles are released at specific sites termed Active Zones (AZ). Two modes of synaptic transmission are known, evoked transmission coupled to action potentials and spontaneous transmission. To investigate spontaneous vesicle fusion at individual AZs, we used transgenic Drosophila flies expressing GCaMP5 tagged to the postsynaptic reticulum. This newly developed line allows us to distinctively observe single synaptic vesicle release events at individual AZs. Statistical analysis of transmitter at individual AZs demonstrated that release in the majority of AZs (84%) fits the Poisson distribution, suggesting that these AZs are all approximately equivalent and have low release probability (LPAZ, Pr=0.006 1/s). However, a small sub-population of AZs (16%) deviated from the Poissonian law and had significantly higher average release probability (Pr=0.33 1/s). Importantly, the small population of HP! AZs accounts for to over 50% of all the release events. Furthermore, the intervals between release events at HPAZs are not randomly distributed. Instead, vesicles at HPAZs tend to be released by bursts with an interval of approximately one second between events.

Abstract 18

SEX DIFFERENCES IN SYNAPTIC PLASTICITY OF THE DORSAL HIPPOCAMPUS AFTER CHRONIC ISOLATION STRESS

Raura Doreste-Méndez, Dinah Ramos-Ortolaza, and Annelyn Torres-Reverón

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The hippocampus is a highly plastic structure that inhibits hypothalamic pituitary adrenal axis via afferents to the hypothalamus. We aimed to assess the effects of social isolation in anxiety and depressive behaviors and in changes in the expression of hippocampal synaptophysin and vesicular glutamate transporter 1 (VGLUT1) in male and female rats. We found increased depressive-like behaviors in isolated versus paired males. Females at midlevel hormones (estrus) showed less anxiety in comparison to low hormone (diestrus) and high-hormone (proestrus) rats. We found an increase in percent area occupied by synaptophysin in CA1 and a trend towards increase in CA3 in isolated versus paired housed male rats. In contrast VGLUT 1, was not different in CA3 but a trend towards increase was found in DG for the paired rats compared to isolated rats. In isolated females, synaptophysin showed fluctuations across the estrous cycle but paired females showed a stable pattern. These results suggest that hippocampal areas are differentially sensitive to chronic isolation. The synaptic stability in paired females across the cycle may be a protective effect against the detrimental effects of stress.

Abstract 19

AUDITORY LEARNING DOWN-REGULATES INHIBITORY SYNAPTIC FUNCTION IN THE SENSORY STRIATUM

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University of Puerto Rico PRISE

Associative learning requires a neutral sensory stimulus paired with an aversive event or reward in order to establish auditory-guided behaviors. This type of learning affects regions of the brain such as the striatum. However, nothing is known about the role of the sensory striatum in auditory learning. To determine whether changes occur within synapses as a consequence of auditory learning we assessed inhibitory synaptic function in the sensory striatum. Gerbils (postnatal day P88-P115) were trained on an amplitude modulation AM discrimination task. On alternate days of training we generated Corticostriatal brain slices that preserved the ventral medial geniculate nucleus with projections to the auditory cortex and layer 5 projections into auditory recipient regions of the striatum. Spontaneous inhibitory postsynaptic currents (IPSCs) and minimum-evoked IPSCs were recorded in medium spiny neurons (MSN) within auditory striatum. Diminished amplitudes of sIPSCs and mE-IPSCs accompanied learning an auditory task and lasted several days before returning to control levels. Changes in sIPSC amplitudes were observed to significantly correlate with changes in task performance. Therefore, a transient period of disinhibition in MSN within the auditory striatum could mediate auditory learning.

Abstract 20

SYNAPTIC VESICLE RECYCLING IS ENHANCED IN COMPLEXIN DEFICIENT DROSOPHILA NEUROMUSCULAR JUNCTIONS

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Complexin Cpx is a small cytosolic protein that interacts with the SNARE complex and regulates the final stages of exocytosis Cpx null mutation in Drosophila produces drastic enhancement in spontaneous synaptic activity suggesting that Cpx prevents spontaneous exocytosis To understand how synaptic vesicle recycling can compensate for this increased activity in Cpx null mutants we combined activity dependent loading of the fluorescent marker FM1-43 with electron microscopy analysis First we tested whether evoked and spontaneous release components in cpx neuromuscular junctions NMJs are associated with different recycling pools of vesicles The NMJs were loaded with the dye either in the absence of stimulation passive ! staining or during electrical stimulation at a 5 Hz frequency active staining and unloaded in the absence of stimulation passive destaining We found that the recycling pool of vesicles was significantly increased in cpx NMJs and that the fluorescence loss during passive destaining was similar at the terminals loaded actively or passively This result suggests the recycling pool is increased in cpx terminals to compensate for the enhanced spontaneous release and that the recycling pools of vesicles participating in the evoked and spontaneous release components are intermixed. To test this hypothesis further, we employed photoconversion of FM1-43 loaded passively at different times ranging from 10 s to 10 min. We found that the number of FM1-43 loaded synaptic vesicles in *cpx(-)* NMJs was significantly increased at every time point and that the recycling and resting vesicles were spatially intermixed. These results suggest that the vesicle pools involved in evoked and spontaneous recycling pathways are mixed in the *Drosophila* NMJs and that *cpx(-)* synaptic boutons have an increased recycling p ool to enable elevated spontaneous fusion.

Abstract 21

EFFECTS OF TEMPERATURE ON SLEEP REGULATION IN THE FRUIT FLY DROSOPHILA MELANOGASTER

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Investigating the sleep pattern of humans living on hot dry climates indicates that hot temperatures are associated with fragmented nighttime sleep and daytime sleepiness. Although our understanding of the sleep circuits has dramatically increased, the mechanisms for temperature regulation of sleep remain elusive. When wild-type flies are assayed at 29°C, they have reduced nighttime sleep but increased daytime sleep compared to 25°C. The increase in daytime sleep has 2 components: 1) one that takes place at the beginning of the light period and depends on the amount of sleep on the previous night (homeostatic component); and 2) one that takes place in the second half of the light period and is independent of sleep history. In addition, we found that the homeostatic component depends on the translational repressor Pumilio within the circadian circuit. Our findings indicate that the circadian circuit is an important player in these effects. Given the importance of sleep in the general health of organisms, understanding how temperature affects sleep is critical to evaluate the impact of global warming.

Abstract 22

CHARACTERIZING MUTATIONS AND THE PHENOTYPE OF MICE WITH DELETIONS IN GTF2I, GTF2IRD1 AND GTF2IRD2

Jesús Acevedo, Nathan Kopp, and Joseph Dougherty

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William's syndrome is a developmental condition characterized by a combination of neurocognitive, physical abnormalities and hypersociability. This disorder is caused by hemizygous deletions of 28 genes on chromosome 7q11.23. GTF2I, GTF2IRD1 and GTF2IRD2 are genes that could be implicated in the social phenotype of this disease. We expect mutant mice to have a hypersocial behavior by having more calls compared to wild type mice. To test the effect of mutations in these genes, we introduce INDELs in mice embryos and genotyped the founder population. We identified 29 out of 57 mice that have a deletion in at least one gene and recorded the ultrasonic vocalizations of the F1 progeny of the founder with mutations in all three genes. We found no difference between the call number in the ultrasonic vocalization experiments between the mice with mutations in GTF2I and GTF2IRD2, and mice with mutations in GTF2IRD1. This work has helped us to characterize the mutations in the founder population, and provided us with preliminary data about the social behavior in these new mutant mice.

Abstract 23

THE ROLE OF EYA2 IN REGENERATION OF THE LATERAL LINE IN ZEBRAFISH

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Interested in the molecular genetics of sensory tissue regeneration, we are focusing on hair cells (HC), mechanoreceptors central to hearing in vertebrates. Contrary to mammals, fish and birds are able to regenerate HC. Fish have a primitive inner ear comparable to the mammalian ear but additionally they have an external sensory structure called the lateral line (LL) composed of sensory patches referred to as neuromasts (N). In each N HC get exposed to the surrounding water movements, transducing them into an electric signal transmitted to the brain via the peripheral nervous system. When destroyed by waterborne copper, HC regenerate from surrounding supporting cells (SC). We propose to assess the possible role of the eya2 gene in HC regeneration. The eya gene family is crucial in the development of sensory tissues and eya2 is abundantly expressed in SC into adulthood. We developed a stable mutant transgenic fish line carrying a construct expressing GFP under the control of the endogenous eya2 promoter thus creating a mutation in this gene. We expect eya2 mutants to show a defective regenerative program.

Abstract 24

THE TRANSCRIPTION FACTOR ENGRAILED ALTERS THE SPECIFICITY OF SYNAPTIC CONNECTIONS OF DROSOPHILA AUDITORY NEURONS WITH THE GIANT FIBER

Adeline Pezier, Sami H. Jezzini, Bruno Marie, and Jonathan M. Blagburn

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Engrailed is a transcription factor with a highly conserved role in neuronal development. We are investigating its role in the formation of the synapse between sound-detecting Johnston's Organ neurons (JONs) and the giant fiber (GF) escape neuron in the fruit fly Drosophila melanogaster. We show that a subset of JONs which express Engrailed form mixed electrical and chemical synaptic inputs onto the GF dendrite. These synaptic connections are detected by trans-synaptic Neurobiotin transfer and by colocalization of Bruchpilot-short puncta. We then show that misexpressing Engrailed in a second subset of sound-responsive JONs causes them to form ectopic electrical and chemical synapses with the GF. Finally, through indirect electrophysiological recording of the synapse, we found that ectopic presynaptic expression of Engrailed strengthens the synaptic connection while RNAi-mediated knockdown of Engrailed reduces the synaptic strength at the JON–GF synapse. Overall, these results suggest that Engrailed in JONs regulate both neuronal excitability and synaptic connectivity. We are now investigating which gap junctions are expressed at this synapse.

Abstract 25

GLUTAMATERGIC AND PEPTIDERGIC TERMINALS DISPLAY DIFFERENT EXO/ENDOCYTOSIS RATES

Liz Marie Bonet[1], [3] and Ramón A Jorquera[1], [2]

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At chemical synapse, the exocytosis occurs by vesicle fusion and release of neurotransmitter where the vesicle may full collapse with the plasma membrane before endocytosis. Kiss-and-Run, a modality of vesicle fusion, occurs by short fusion episodes without full collapse, but with retraction. This type of fusion is present in the hippocampus and in Peptidergic terminals. The neuromuscular junction of Drosophila at muscles 6/7 are innervated by Glutamatergic synapses, but, the neighboring junctions at muscles 12/13 are innervated as well by Peptidergic neurons. We compare the exo/endocytosis properties between Glutamatergic and Peptidergic terminals by uptake and release of FM_1-43 dye at muscles 6/7 and 12/13. Imaging experiments were performed under a laser scanning fluorescent microscope. Our results indicate similar profiles of dye uptake between both terminals, however, the fluorescent brightness remaining after the exocytosis procedure was larger in Peptidergic terminals. These results suggest a possible Kiss-and-Run mechanism of fusion of Peptidergic terminals at the neuromuscular junction of Drosophila larvae.

Abstract 26

SYNAPTOTAGMIN-7 IS NOT THE NERVE-EVOKED ASYNCHRONOUS CA2+-SENSOR BUT CONTROLS DELAYED RELEASE

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At chemical synapses, nerve-stimulation evokes two major forms of releases, the fast synchronous and the slow asynchronous. A much slower asynchronous form, delayed release, is induced during high-frequency nerve-stimulation, decaying after the stimulation cease. Asynchronous releases are thought to be important for postsynaptic spikes emergence. Nevertheless, how asynchronous releases are regulated remain unknown. Recently, Synaptotagmin-7 (Syt7) was proposed as the asynchronous calcium-sensor, however, it`s still under debate. We confirmed by RT-PCR a Drosophila P-element insertion line to abolish Syt-7 gene transcription (Syt7-/-). Two-electrode voltage-clamp was used to analyze the release properties of Syt7-/-. Our results indicates that Syt7-/- did not alter the basal synaptic transmission, but burst of high-frequency nerve-stimulation failed to induce the asynchronous delayed release observed in control. Intriguingly, the asynchronous release evoked by a single nerve-stimulus remains in Syt-7-/-. These results indicates that Syt-7 is not the asynchronous calcium-sensor for the single evoked release but is critical for asynchronous delayed release.

Abstract 27

NEURAL CORRELATES OF CONDITIONED FEAR RETRIEVAL IN THE PARAVENTRICULAR THALAMUS

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Pharmacological inactivation of the dorsal midline thalamus (dMT) impaired fear retrieval when performed 24h after fear conditioning, but not 2h after. These results suggest that one or more structures within dMT are recruited into the fear circuit after conditioning. Consistent with this, the paraventricular nucleus of the thalamus (PVT), a subregion of dMT, showed increased expression of the neural activity marker cFos 24h after conditioning, but not 6h after. cFos measurements are limited because they cannot track the same neurons at different time points. We therefore used single unit recording to track PVT neurons before, 2h after, and 24h after fear conditioning. Regarding spontaneous firing rate, more neurons showed increases 24h after conditioning (51%), compared to 2h after (16%), consistent with cFos findings. In contrast, the percentage of cells showing conditioned tone responses ($Z > 2.58$ in the first two seconds after tone onset) was the same at both 2h and 24h time points (16%). Interestingly, the neurons that were tone-responsive at 24h were not tone-responsive at 2h, and vice versa, suggesting that distinct PVT ensembles may be recruited over time. Thus, time-dependent changes in both spontaneous and tone-induced firing are consistent with time-dependent recruitment of PVT neurons for retrieval of conditioned fear.

Abstract 28

DEEP BRAIN STIMULATION OF THE VENTRAL STRIATUM FOR THE EXTINCTION OF MORPHINE PLACE PREFERENCE

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Deep brain stimulation (DBS) is a neurosurgical procedure used to treat refractory neuropsychiatric disorders. Recent studies have suggested that DBS of the ventral striatum (VS) may be a potential target for the treatment of addiction. Despite these results, DBS parameters, electrode placement, and the addiction stage have not been thoroughly addressed. In this study, we examined whether DBS of the VS could facilitate the extinction of morphine-induced conditioned place preference (CPP). For this purpose, male rats were stereotaxically implanted with bipolar electrodes aimed at the VS. Using CPP paradigm, rats were conditioned to prefer the morphine-paired side. Subsequently, rats expressing morphine-CPP received extinction sessions, together with high-frequency DBS (HF-DBS), low-frequency (LF-DBS) or sham stimulation. We found that HF-DBS impaired the extinction of morphine-CPP. On the other hand, LF-DBS facilitated the extinction of morphine-CPP even after the DBS was turned off. These results suggest that LF-DBS of the VS might represent a promising therapy for opioid addiction.

Abstract 29

ACTIVITY-DEPENDENT INHIBITORY RELEASE REGULATED BY SYNAPSIN II AND RAB3A AT HIPPOCAMPAL SLICES

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Synapsins are a family of phosphoproteins localized to synaptic vesicles, and synapsin deficiency produces epilepsy. The epileptic phenotype observed in synapsin II knockout SynII(-) animals may result from a deficit in inhibitory transmission. Since epileptic seizures in SynII(-) mice are usually associated with overexcitability at the hippocampus, we investigated synchronous and asynchronous inhibitory release components at hippocampal slices of SynII(-) mice by performing paired recordings. We found that SynII deletion increases and synchronizes GABA release in response to a single action potential. In contrast, the asynchronous release component evoked by high frequency stimulation was significantly reduced in SynII(-) neuron, and this effect was specific to the neuron subtype. This surprising observation, suggests that Synapsin II may play a distinct role in regulating the timing of GABA release at particular subtypes of hippocampal interneurons. Since Synapsin II deficiency promotes epileptic seizures, we suggest that a reduction in GABA asynchronous release at a specific population of inhibitory interneurons may play an important role in the epileptic phenotype observed in Synapsin II deficient animals.

Abstract 30

HONEY BEES PRESENT SHIFT WORK IN FORAGING AND FANNING TASKS.

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Although shift work is an essential component in human societies, it has been associated with circadian misalignment and increased susceptibility to many diseases. A study investigating if pollen foragers captured during the morning were genetically different from those captured in the afternoon found that there are paternal lineages that are only observed either in the morning or the afternoon, suggesting that that shift work may occur in pollen foragers. Based on these findings we hypothesized that honey bees exhibit shift work in foraging task. To test this we performed direct observations of entry, exit, pollen load and fanning behavior for 14 days. Consistent with our hypothesis, we found that 18% of individuals only foraged in the morning and 26% foraged in the afternoon, while the remaining bees foraged during both observation periods. In addition, some individuals change shifts as they age while others remain in the same shift morning or afternoon. Surprisingly, we found that fanning task is also performed in shifts. Analyzing the relationship of foraging and fanning shifts revealed that they are related but separable tasks.

Abstract 31

THE EFFECT OF DEVELOPMENTAL EXPOSURE OF ALCOHOL ON THE TIMING OF DEVELOPMENT, SLEEP STRUCTURE, AND NEUROPEPTIDE CELL MORPHOLOGY IN DROSOPHILA MELANOGASTER

D. Giray; **R.A. Aponte Rivera**; P.M. Cruz Vazquez; A. Cotto Aponte; J. R. Sanchez Gonzalez; J. Aleman Rios; N. Rodriguez; M.G. Echevarria Guadalupe; K.E. Torres Torres; J. Aponte; N. Cruz Bermudez; and J.L. Agosto-Rivera

University of Puerto Rico Río Piedras

The consumption of alcohol during pregnancy is associated with a high risk of abortion, perinatal mortality, congenital defects, and sleep alterations in the offspring. It has been estimated that 11% of pregnant women in the USA consume alcohol during pregnancy while in Puerto Rico, 32% of women consume alcohol during pregnancy. However, the mechanism by which alcohol affects development and its consequences on adult behavior are unclear. Here we used Drosophila as a model system and examined the effects of developmental exposure of alcohol on the timing of development, sleep structure, and the morphology of the circadian neurons. Our results indicate that early exposure of alcohol delays development and fragments sleep in the adult. The results of circadian cell morphology will also be examined and presented. These findings may provide insight into the mechanisms underlying the developmental effects of alcohol in humans.

Abstract 32

MODULATION OF FKBP5 AFFECTS FEAR CONDITIONING AND EXTINCTION

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Ponce School of Medicine and Health Sciences

Current studies associate FK binding protein 5 (Fkbp5) variants with PTSD. Since Fkbp5 decreases the activity of the glucocorticoid receptor (GR), excessive Fkbp5 expression could reduce GR activity and impair extinction memory. In this study, we hypothesized that Fkbp5 in the infralimbic cortex (IL) plays an important role in fear extinction by modulating GR activity. To test this, adult male rats were exposed to fear conditioning and extinction. Then, IL tissue punches were extracted to determine mRNA changes. Fkbp5 mRNA expression increased after fear conditioning and decreased after fear extinction suggesting that Fkbp5 modulates extinction. To examine this, we infused Fkbp5-shRNA or control plasmids into IL to downregulate Fkbp5 expression prior to fear conditioning and extinction. Unexpectedly, rats infused with Fkbp5-shRNA acquired less fear during conditioning which was maintained during fear extinction and extinction recall. In contrast to most studies in IL, our results suggest that Fkbp5 levels in IL modulate fear acquisition and extinction. Our findings also suggest that Fkbp5 variants which lead to increased expression of Fkbp5 in the prefrontal cortex may predispose patients to the development of PTSD.

Abstract 33

CONTROL OF CORTACTIN LEVELS BY WNT CONTRIBUTES TO RAPID ACTIVITY DEPENDENT SYNAPTIC PLASTICITY

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Major signaling molecules, such as Wnt, initially characterized as key early developmental regulators are also essential for the plasticity of the nervous system. A challenge remains to understand how these potent signals are transduced into the necessary cellular changes underlying this plasticity. Here we focus on the actin regulator Cortactin and define Cortactin's new role in synaptic plasticity.

We use genetics, confocal microscopy and electrophysiological recordings to assess the role of Cortactin in regulating the modifications in synaptic structures and function occurring at the Drosophila neuromuscular junction (NMJ) after repeated stimulations. We show that Cortactin is present pre and post-synaptically at the NMJ and that pre-synaptic Cortactin is necessary for the rapid activity-dependent modifications in synaptic structure and function. In addition, we show that Cortactin level is increased at stimulated synaptic terminals and that this increase requires de novo transcription and depends on Wnt expression. We argue that the dynamic expression of Cortactin during repeated stimulation protocol makes it a key molecular component regulating synaptic plasticity under the control of the Wnt signal.

Funding: NIH-NIGMS 1P20GM103642

Abstract 34

THE AUTOPHAGY-HIGHWIRE-MAPKKK COMPLEX REGULATES THE TEMPERATURE-DEPENDENCE OF SYNAPTIC GROWTH AT THE DROSOPHILA NMJ

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Human activities have contributed enormously to the increase of the mean temperature of the planet. What are the effects of these changing temperatures on poikilotherms? At the Drosophila NMJ, we observed a linear increase in synaptic growth with increasing temperatures (15°C, 20°C, 25°C and 30°C) and found a spectacular 130% increase in synaptic growth between animals raised at 15°C and 30°C. Here we report the identification of molecules underlying the temperature-dependence of synaptic growth. We show that the Autophagy-Highwire-Mapkkk complex is used to control synaptic size at different temperatures. We identified the highwire gene (hiw) and its inhibitors, the autophagy genes (atg) to be required for the temperature-dependence of synaptic growth. Indeed, hiw mutants show temperature-independent synaptic overgrowth while autophagy mutants show temperature-independent synaptic undergrowth. In addition, we show that a MAPKKK gene is necessary for the increase of s! ynaptic growth at 30°C. Finally, we propose a mechanism for the temperature-dependence of synaptic growth where autophagy is more activated at 30°C than at 15°C, leading to different accumulations of highwire protein and different synaptic growth.

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Abstract 35

CONTROLLED CORTICAL IMPACT BEFORE OR AFTER FEAR LEARNING DOES NOT AFFECT FEAR EXTINCTION IN MICE

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Post-traumatic stress disorder (PTSD) is characterized in part by impaired extinction of conditioned fear. Traumatic brain injury (TBI) is thought to be a risk factor for development of PTSD. We tested the hypothesis that controlled cortical impact (CCI) would impair extinction of fear learned by Pavlovian conditioning in mice. To mimic the scenarios in which TBI occurs prior to or after exposure to an aversive event, unilateral (uCCI) was delivered to the parietal cortex at one of two time points: 1) prior to fear conditioning, or 2) after conditioning. Delay auditory conditioning was achieved by pairing a tone with a foot shock in context A. Extinction training involved the presentation of tones in a different context (context B) in the absence of foot shock. Test for extinction memory was achieved by presentation of additional tones alone in context B over the following two days. In pre- or post-injury paradigms, uCCI did not influence fear learning and extinction. Furthermore, uCCI did not affect locomotor activity. Our results demonstrate that uCCI does not impair the acquisition and expression of conditioned fear nor of extinction memory.

Abstract 36

COMPLEXIN NEURONAL OVEREXPRESSION ALTERS DROSOPHILA BEHAVIORS

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Complexin binds the machinery for synaptic vesicle exocytosis, preventing spontaneous fusion and promoting nerve-evoked neurotransmitter release, crucial for computation and motor performance. However, the role of complexin at the nervous system is not clear. Loss of complexin in animals induces infertility, loss of vision, ataxia and early lethality. Recent evidence suggests that complexin levels at the nervous system change during pathophysiology or trauma where motor performance and cognition has been affected. In addition, altered complexin levels and transport have been observed in Huntington and Schizophrenia disease models. In order to scrutinize the effect of increased complexin levels in the nervous system, we look into several behaviors in adult Drosophila with complexin trans-gen over-expression in neurons. Climbing, geotaxis, phototaxis and lifespan were assayed. Over expression of complexin decreases the lifespan and increases the basal activity of the animals at early age. Climbing was reduced dramatically after 25 days and abolished this behavior after 45 days. Animal between 7-12 days display reduced geotaxis and abolished phototaxis.

Abstract 37

ULTRASTRUCTURAL CHANGES IN THE OPTIC NERVE AFTER INJURY AND NEUROTROPHIC FACTOR TREATMENT

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We have shown that ciliary neurotrophic factor (CNTF) and fibroblast growth factor (FGF-2) have strong facilitatory effects on axon regeneration in the adult frog optic nerve after injury. In these nerves, bundles of regenerating axons were associated with astrocytes and macrophage-like cells. The objective of the present study is to characterize the identity of these cells, and to determine the changes that occur after CNTF or FGF-2 application. Electron microscopy was used to characterize the ultrastructure and localization of the macrophages/microglia after injury and neurotrophic treatment. We found a large number of macrophage-like cells at the lesion site, and distally in close proximity to regenerating axons in CNTF and FGF-2 treated nerves. Many of these macrophage-like cells at, and distal to, the lesion were ED-1-positive in both CNTF and FGF-2 treated nerves. Both M1 (Arginase-positive) and M2 (CD-86-positive) subtypes were identified. In conclusion, the application of CNTF and FGF-2 affects the number and the distribution of macrophage subtypes after injury and during optic nerve regeneration.

Abstract 38

CHARACTERIZATION OF NEURONAL AND NEUROENDOCRINE CELLS IN THE LUMINAL EPITHELIUM OF HOLOTHURIA GLABERRIMA

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University of Puerto Rico Río Piedras, Developmental Neurobiology Laboratory

Echinodermata is a phylum of marine animals that lie at the basal branch of the deuterostome evolutionary branch. They have a radial morphology that includes a radial nervous system. Recent work by our laboratory and others have explored the echinoderm nervous components. Here we evidence the neurosecretory component of their digestive system. Immunohistological techniques, were used to identify the neuroendocrine cells in 4 digestive tract regions: the esophagus, the descending small intestine, ascending small intestine, and the large intestine. In particular, the antibodies RN1, anti-GFS, a-Galanin, a-GABA and a-Nurr1 were used to observe, describe and quantify the neuroendocrine cells. Different morphologies, abundance and locations suggest that these neuroendocrine type cells are two different subpopulations. Labeling with other antibodies is being done to clearly define the complete catalogue of neuroendocrine cells in the digestive system. The results obtained through these studies will provide important information on echinoderm anatomy, nervous system connectivity, and for future studies of cellular differentiation during intestinal regeneration.

Abstract 39

RETINOIC ACID AND RETINAL GANGLION CELL SURVIVAL AFTER OPTIC NERVE INJURY.

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Recovery of vision after optic nerve injury requires retinal ganglion cell RGC survival axonal regrowth past the area of the lesion and reformation of appropriate synaptic targets The adult mammalian visual system regenerates poorly and past therapeutic efforts have enabled regrowth of only a small number of retinal axons Frog RGCs on the other hand suffer an approximately 50 cell loss after injury but regeneration and reconnection to target areas still occur because of the lack of inhibitory glial molecules and physical obstructions This makes them a good model to explore which molecules influence RGC regeneration Understanding what occurs after RGC injury is of great medical importance because it results in blindness after optic nerve injury and also in conditions such as glaucoma diabetes and optic ischemia The focus of this work is to understand how the activity of retinoic acid RA signaling known to exert effects on neurite outgrowth during development and homeostatic synaptic plasticity in the brain affects RGC survival and the activation of important intracellular signaling pathways

Abstract 40

ACTIVATION OF INNATE IMMUNE SYSTEM IMPAIRS AUDITORY FEAR EXTINCTION

Maria M. Quinones, Bethzaly Velazquez, Lizette Maldonado, and James T. Porter

Ponce School of Medicine

Patients with post-traumatic stress disorder (PTSD) tend to show signs of a relatively increased inflammatory state suggesting that activation of the immune system may contribute to the development of PTSD. We tested whether activation of the innate immune system can disrupt acquisition or recall of auditory fear extinction using an animal model of PTSD. Male adolescent rats received auditory fear conditioning. The next day, an intraperitoneal injection of lipopolysaccharide (LPS) prior to auditory fear extinction impaired both acquisition and recall of extinction suggesting that activation of the immune system impaired extinction learning. LPS after extinction training did not impair extinction recall suggesting that LPS did not affect consolidation of extinction. Furthermore, contextual fear extinction was not affected by prior injection of LPS. Although LPS also reduced locomotion, we could dissociate the effects of LPS on extinction by using a lower dose of LPS which impaired locomotion without affecting extinction. In adult rats, extinction learning and recall were impaired 15 hrs after an injection of LPS without affecting locomotion. Our results suggest that immune activation disrupts learning of auditory fear extinction.

Abstract 41

ACTIVATION OF PHOSPHOLIPASE C IS THE LINK BETWEEN ACETYLCHOLINESTERASE INHIBITION BY ORGANOPHOSPHOROUS NERVE AGENTS AND BRAIN DAMAGE

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Universidad Central del Caribe

Acute poisoning with organophosphorous inhibitors of acetylcholine esterase (AChE) causes an accumulation of acetylcholine (ACh) and overstimulation of the muscarinic receptors. The supraphysiological muscarinic activity causes glutamatergic seizures and excitotoxicity responsible of the majority of the brain injury caused by organophosphates. The link between muscarinic overstimulation and glutamate dependent brain damage is not known. Scrutiny of current literature suggested that the activation of muscarinic M1 receptors coupled to Gq protein activates phospholipase C (PLC). PLC depletes the pool of membrane phosphatidylinositol 4,5-bisphosphate (PI-4,5-P) required for the activity hyperpolarizing M-current. Decreased activity of M currents leads to neuronal depolarization that results in glutamate release and excitotoxicity. Here we test the hypothesis that the inhibition of the PLC leads to reactivation of M currents and prevents the excitotoxicity. Our main finding was that the inhibition of PLC 30 min after application of DFP precludes the damage. Suggesting that the inhibition of PLC the pool of (PI-4,5-P) is replenished and the M- currents reestablished.

Abstract 42

LOCOMOTOR ACTIVITY AND ELECTROPHYSIOLOGY TO ASSESS THE EFFECTS OF CONTAMINANTS ON FRESHWATER PRAWN NERVOUS SYSTEM

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Rico Center for Environmental Neuroscience

Urbanization in Puerto Rico in the metropolitan area and the resulting anthropogenic activities have an impact on the quality of water resources. We seek to understand how this dramatic level of urban development has impacted the rivers in this area and how these changes affect the behavior and nervous system of aquatic fauna. Here we show how freshwater prawn larvae can be used to assess the effects of water contaminants on locomotion and animal´s activity during alternating cycles of light and darkness. Two species of prawns, Macrobrachium rosenbergii and M carcinus, showed opposite responses to the light/dark cycles, the latter showing a decrease in locomotor activity after exposure to low levels (0.006 mg/L) of dibutyl phthalate, but not of chromium (0.100 mg/L). We also present preliminary data on how the prawn's neuromuscular junction may be used to assess potential effects of urban river contaminants on synaptic function.

Abstract 43

HETEROGENEOUS SYNAPTIC GROWTH AT THE DROSOPHILA NEUROMUSCULAR JUNCTION

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Universidad Central del Caribe

Drosophila neuromuscular junction provides an excellent model system to study activity-dependent formation of new synapses, since the growth of new synaptic boutons in this preparation can be robustly and rapidly induced by patterned depolarization. We investigated the formation of new boutons combining confocal imaging and EM tomography. We used a green fluorescent protein (CD8-GFP) tag to monitor the outgrowth of new boutons.and mCherry tag for a synaptic vesicle protein, Synaptogyrin (Gyr-mCherry). We identified two types of new boutons – those that contained synaptic vesicles, and those devoid of vesicles. The boutons of the second type were filled with filaments and membrane structures. The boutons devoid of vesicles did not either acquire vesicles or degrade within an hour, and additional stimulation did not change this pattern.

Abstract 44

THE ROLE OF GUT MICROBIAL COMMUNITIES ON THE SLEEP PATTERNS OF DROSOPHILA MELANOGASTER

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The advances of high throughput sequencing have made it possible to identify diversity and abundance of the microbes that make up symbiotic microflora. Various studies have found that the gut microbiota can have an impact on their host's behavior. Here we propose that gut microbial communities play a role in the regulation of sleep behavior and that sleep behavior helps the host to maintain healthy levels of intestinal microbes. To test this hypothesis, we took advantage of the knowledge of sleep behavior and gut microbiota in the fruit fly, Drosophila melanogaster. We found that when flies are expose to an antibiotic treatment during development, sleep was decreased by 16%. In contrast, antibiotic treatment only at the adult stage exhibit a 13% increased in sleep. Metagenomic analysis indicates that the composition of the gut microbiota changes between day and night. These results suggest that gut microbiota is important for sleep patterns.

Abstract 45

SITE-DIRECTED RNA EDITING

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Adenosine Deaminases that Act on RNA ADARs are a conserved family of enzymes that catalyze a natural process of site-direct mutagenesis. Biochemically they convert adenosine to inosine a nucleotide that is read as guanosine during translation; thus when editing occurs in mRNAs, codons can be recoded and the changes can alter protein function. By removing the endogenous targeting domains from human ADAR2 and replacing them with an antisense RNA oligonucleotide, we have engineered a recombinant enzyme that can be directed to edit anywhere along the RNA registry. We hypothesize that our system could be used to correct genetic mutations or manipulate protein function. As proof of principle in vitro, we correct a premature termination codon in mRNAs encoding the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) anion channel. In Xenopus oocytes we show that a semi-genetically encoded version of our editase can correct CFTR mRNA, restore full-length protein and reestablish functional chloride currents. Finally, in a human cell line we show that a fully-genetically encoded version can correct a non-functional fluorescence reporter. Recent improvements to our system show that we can restore about a third of the reporter's function in cellula.

Abstract 46

ZEBRAFISH: SCREENING TOOL FOR WATERBORNE POLLUTANTS IN PUERTO-RICAN RIVERS

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University of Puerto Rico Medical Sciences Campus

To monitor deleterious effects of waterborne pollutants on the nervous system of local river fauna we established an approach in a laboratory animal model, zebrafish. We have established a quantitative behavioral assay in larvae which we will ultimately link to the genetic network implicated in the elaboration of this behavior. We measured swimming activity during light/dark cycles and assessed place preference of untreated animals versus animals treated with a first chosen compound, copper. At C1X, (maximum contaminant level goal established by the EPA for drinking water) larvae were hyperactive and their place preference was affected. Furthermore, we found that copper-treated animals increased their activity when compared to untreated larvae when the light was turned on, but reacted the same when light was turned off. This implies that those two responses function with two different underlying neuronal circuitries with the first one only being sensitive to copper. Next, we want to test selected mutants that we are currently generating using the CRISPR-Cas technology in this behavioral assay. We are raising founders to test their offspring and assess the involvement of each selected gene in the observed altered behaviors.

Abstract 47

LOCALIZATION OF BGNPY-LIKE IMMUNOREACTIVITY IN THE NERVOUS SYSTEM OF BIOMPHALARIA GLABRATA, AN INTERMEDIATE HOST FOR SCHISTOSOMIASIS.

Solymar Rolón-Martínez, Nadia Delgado-Rivera, Grace Torres, Lee O. Vaasjo, Elsie Rivera, and Mark W. Miller

University of Puerto Rico Medical Sciences Campus

The digenetic trematode species Schistosoma mansoni that causes the most widespread form of human intestinal schistosomiasis ("snail fever") employs the freshwater snail Biomphalaria glabrata as its primary intermediate host. Previous investigations in other schistosome-snail systems, showed that neuropeptide Y (NPY) gene expression increased during snail infection. This investigation explored the localization of Biomphalaria glabrata NPY (BgNPY) in the central and peripheral nervous systems of the snail. BgNPY-like immunoreactive neurons were present in most central ganglia; buccal ganglion (dorsal: 9 ± 2, ventral: 3 ± 1), cerebral ganglion (dorsal: 17 ± 2, ventral: 19 ± 7). Larger BgNPY-li neurons in the left parietal ganglion (dorsal: 14 ± 5, ventral: 7 ± 4), and visceral ganglion (dorsal: 23 ± 3, ventral: 12 ± 6) had prominent axons oriented toward the parietal-visceral connective. These results suggest that BgNPY could be involved in behaviors such as food intake and reproduction, and are consistent with a role of this neuropeptide in the redirection of energy resources in the Schistosoma mansoni - Biomphalaria host-parasite system.

Abstract 48

CHARACTERIZATION OF MYELINP2 AND SLITRK ECHINODERM GENES DURING ENS REGENERATION

Ernest Monahan-Vargas, James Otero, and José E. García-Arrarás

Institution(s) requested

Holuthuria glaberrima is an echinoderm known for its ability to regenerate its internal organs including its enteric nervous system (ENS). Unfortunately, there is a limited amount of neural markers and many of the molecular events that occur during the regeneration of this system are still unknown. We identified two genes that might be associated with regeneration: slitrk and myelinP2. Slitrk proteins have been shown to modulate neurite outgrowth. MyelinP2 protein is a component of the myelin sheath in vertebrate glial cells, and holothurian glia has been shown to play an important role in holothurian CNS regeneration. We have shown that these genes are expressed in normal and regenerating sea cucumber using bioinformatic tools, sqPCR and immunohistochemistry. To view the localization of MyelinP2 protein, we developed an antibody which localized it to the ceolomic epithelium of the intestine and muscle. Finally, our work also explores the evolution of myelin-related proteins, an interesting development from our findings, since myelin is not found in invertebrate nervous systems. In conclusion, understanding the role of these genes may aid in determining patterns of ENS regeneration of H. glaberrima.

Abstract 49

MORPHINE WITHDRAWAL REVEALS SEX DIFFERENCES IN CONTEXTUAL RESPONSES DURING CUED FEAR CONDITIONING AND EXTINCTION

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Sex difference has been show in drug abuse and addiction. Previously we had found that withdrawal from chronic morphine produced a deficit in cued fear extinction learning in male rats but not in females. Thus, we aimed to elucidate if fear response (freezing) to the conditioning context was also different by sex. Rats were exposed to chronic morphine injections and somatic signs of spontaneous withdrawal were assessed. Cued fear conditioning started 84 hours after the last morphine injection and consisted of three phases: conditioning, extinction and the test. Contextual fear was measured by analyzing a 1-min pre-tone period. Morphine treated males and ovariectomized females showed a tendency for increased freezing immediately upon exposure to the chamber on extinction but cycling females did not freeze significantly to the context independent of the treatment. This suggest that in males and ovariectomized females, withdrawal alters fear expression independent of the withdrawal context, but when ovarian hormones are present, fear responses might depend on re-exposure to the withdrawal context.

Abstract 50

ALTERED SYNAPTIC VESICLE RECYCLING AT COMPLEXIN KO.

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The availability and trafficking of synaptic vesicles is crucial to sustain neurotransmission during periods of high demand. Complexin binds and stabilizes the SNARE-complex fusion machinery, generating superprimed vesicles. Complexin levels regulate the availability of the ready-releasable pool of vesicles, in agreement with Complexin's function in priming; however its role in vesicle trafficking is unclear. Here, we investigate the role of Complexin in vesicle trafficking. We correlate voltage-clamp recordings with exo/endocytosis fluorescent imaging at Drosophila neuromuscular junction. Vesicle pools size and recycling were estimated in conditions where the endocytosis was blocked with Dynasore. In the absence of calcium influx, large spontaneous recycling was observed in Complexin null as in wild-type with sucrose, indicating exocytosis induced recycling. Stimulus-evoked recycling and ready-releasable vesicles were decreased in nulls. Time-course of quantal release and fluorescent distaining were slower in nulls, consistent with vesicle priming alterations. Our work indicates that priming defects limits recycling during nerve-evoked activity but not spontaneously.

Abstract 51

INFRALIMBIC CORTEX INACTIVATION IMPAIRS EXTINCTION OF AVOIDANCE; EXTINCTION IS RESCUED BY SUBSEQUENT INTRA-IL BNDF.

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Avoidance is a core symptom of anxiety disorders, and is commonly resistant to behavioral extinction. Decreased activity in ventromedial prefrontal cortex (vmPFC) has been linked to poor extinction retention in anxiety patients. In rodents, deficits in infralimbic cortex (IL, vmPFC homolog in rodents) results in extinction failure as well. Moreover, inactivation of IL impairs fear extinction. We therefore developed a rat model of avoidance extinction failure by pharmacologically inactivating IL prior to avoidance extinction training. We found that rats were able to acquire within-session extinction of, but were not able to retrieve extinction memory subsequently over days, even after drug-free training. Thus, IL inactivation results in impaired avoidance extinction, which resembles pathological avoidance. Because intra-IL infusion of BDNF enhances fear extinction, we tested whether intra-IL BDNF could rescue avoidance extinction. Indeed, intra-IL BDNF reduced both avoidance and freezing spontaneously, suggesting that BDNF enhances extinction of both fear and avoidance. These results suggest that IL is necessary for avoidance extinction and that upregulation of cortical BDNF could rescue avoidance extinction deficits, as it does in fear extinction.

Abstract 52

COMPLEXIN REGULATES SYNAPTIC FILTERING AND INFORMATION ENCODE

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Neurons communicate by patterns of nerve action-potentials and quantal release. Alteration in quantal release critically affects the postsynaptic responses and the action potentials patterns. Complexin regulates the last step of quantal release and has been associated with several neuronal dysfunctions, however its role in synaptic function and diseases is still under investigation. Here we explored the synaptic filtering properties of Drosophila glutamatergic synapses and how Complexin participates. We combined genetic manipulation of Complexin level in live animals, current and voltage clamp recording of postsynaptic activity, pharmacology and signal analyses. Overall Complexin alterations in spontaneous and evoked quantal releases, we found that Complexin null animals display sever defects in the synaptic filtering properties. Spontaneous quantal release in Complexin null synapses display a Direct-Current component during voltage-clamp but not in current-clamp indicating an active resting membrane potential rectification in short-time. Spectral analyses of postsynaptic membrane potential recordings indicate an increase in the low-frequency power-content in nulls animals. These results correlates with supernumerary post-synaptic spikes observed in complexin null during nerve-stimulation.

Abstract 53

THE CESF CLUSTER IN THE PULMONATE SNAIL, BIOMPHALARIA GLABRATA, NEURAL SUBSTRATE CONSERVED ACROSS GASTROPOD MOLLUSKS

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How the brain changes with the gain and loss of behaviors across species is still a mystery. A cluster of five serotonergic neurons on the dorsal side of the cerebral ganglia is present in all Opithobranch: Mollusca tested. In this study, we expand the knowledge of the structure and function of these neurons to include Pulmonates. It is hypothesized that if the anatomy and physiology of the CeSF cluster in the Pulmonate snail Biomphalaria is similar to that reported in Opisthobranch sea slugs then, this cluster is homologous and its conservation across taxonomically distant species may represent an ancestral multi-circuit relation. Results show how morphology, projections, sensory input and synaptic relations to different circuits are conserved. Thus the intertwining of different behaviors such as responses to light, predator avoidance and suppression of feeding may give rise to new behaviors in different mollusks, such as swimming or decision making. This work is important because it expands the taxonomic analysis and understanding of the amazing variability of this cluster to that of Heterobranchia.

Abstract 54

CAFFEINE STIMULATES LOCOMOTOR RHYTHM IN THE MAMMALIAN SPINAL CORD THROUGH AN A1/D1-DEPENDENT MECHANISM

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Caffeine is a strong psycho-stimulant drug socially consumed worldwide and like cocaine and amphetamines it can modulate behaviors such as vigilance, attention, and locomotion. Caffeine is as a non-selective blocker of adenosine receptors, specifically the A1 and A2A subtype. Through the blockade of endogenous adenosine, caffeine disrupts the dopamine homeostasis, which is key for the onset of many psychiatric disorders and drug addiction. We began assessing the effects of caffeine to spinal locomotor network function using the neonatal mouse isolated spinal cord preparation. A locomotor rhythm was obtained by adding a combination of serotonin, N-methyl-D-Aspartate (glutamate analog) and dopamine to the recording chamber and an alternating locomotor-like rhythm was confirmed by recording motor activity using suction electrodes on lumbar ventral roots. Addition of caffeine to the superfusate significantly decreased the step cycle period of the ongoing locomotor-like rhythm, while decreasing burst duration in most preparations in a reversible manner. Our results support the stimulant effects of caffeine onto the spinal network controlling hindlimb locomotion, acting primarily through the inhibition of A1 adenosine receptors, which makes available the D1 dopamine receptor.

Abstract 55

MELANOTRANSFERRIN: NEW HOMOLOG GENES AND THEIR DIFFERENTIAL EXPRESSION DURING INTESTINAL REGENERATION IN THE SEA CUCUMBER HOLOTHURIA GLABERRIMA

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The sea cucumber Holothuria glaberrima represents an excellent model to analyze and characterize the molecular mechanisms associated with the repair and regeneration of the digestive tract. Previous gene expression analyses of distinct intestinal regeneration stages have showed over-expression of certain genes involved in development, growth, cancer andimmune processes. Among these, four different Melanotransferrin (MTf) like genes were sequenced from cDNA libraries of the normal and regenerating intestine and the radial nerves. The four genes were found to be over-expressed during the early stages of intestinal regeneration (3-7 days post evisceration) and at radial nerves. The finding of 4 different genes in the holothurian is particularly surprising, because all other animal species sequenced to date have only one MTf gene. Thus, our findings suggest a new possible function of MTf in organogenesis and in particular in the intestinal and enteric regenerative process.

Abstract 56

NEUROPROTECTION BY 4R-CEMBRANOID AGAINST OXYGEN GLUCOSE DEPRIVATION

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Ischemic stroke is one of the main leading causes of death and disabilities in the US. There are no neuroprotective agents available, leaving the search for new drugs an important segment of research. In the present work, we analyzed 4R-induced neuroprotection using the recovery of population spikes as measurement of functional neurons against oxygen glucose deprivation, considered as an in vitro model of stroke. Hippocampal slices submitted to 10 min of oxygen glucose deprivation (OGD) were superfused with 10µM of 4R immediately or 30 minutes after the initiation of deprivation. We observed a significant recovery of population spikes in both treatments; 27.024± 5.2% (OGD), 56.7 ± 7.2 (immediately after) and 65 ± 7.1 (30 minutes after). Since neuroprotection was observed, a neuroprotective pathway was determined. Wortmannin, a PI3K inhibitor, blocked the neuroprotective effect of 4R showing that this pathway is necessary to induce neuroprotection.

Abstract 57

HISTAMINE IMMUNOREACTIVITY IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEMS OF BIOMPHALARIA SPP., INTERMEDIATE HOSTS FOR SCHISTOSOMIASIS

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Biomphalaria is the intermediate host for the trematode parasite Schistosoma mansoni. Biogenic amines, such as histamine, play important roles in the nervous systems of gastropod snails. Evidence suggests that, asexual transformation of the parasite depends upon uptake of these amines from the snail host. Little is known, however, concerning potential sources of histamine in Biomphalaria or the roles that it might play in the snail. We localized histamine-like immunoreactivity in the central nervous system and peripheral tissues of Biomphalaria. The presence of immunoreactive cells throughout the central ganglia was indicative of diverse regulatory functions. In addition, the foot, tentacles, and other body regions, which are all common sites of S. mansoni miracidium penetration and transformation, were innervated by histaminergic fibers. The activation of the Biomphalaria histaminergic neural system may occur during infection. Comprehensive studies of histaminergic signaling could increase our understanding of host-parasite interactions during larval stages of the schistosome life cycle, which may lead to control strategies for intestinal schistosomiasis.

Abstract 58

MGLUR5 MODULATION WITHIN NAC SHELL DURING ENVIRONMENTAL ELICITED COCAINE CONDITIONING

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The metabotropic glutamate receptor 5 (mGluR5) within the Nucleus Accumbens (NAc) has been implicated in modulating psychostimulant reward. Data from our laboratory demonstrate that mGluR5 blockade decreased environmental-elicited cocaine conditioning response. We focused in elucidating which proteins have been affected during this expression by blocking mGluR5 with MPEP. Rats were implanted with cannula within NAc shell, received systemic injections of saline/cocaine for 10 consecutive sessions, and were exposed to a multimodal environment that signaled cocaine or saline. On the test session (Day 12) separate groups were infused within NAc shell with MPEP. The ratio of pERK/ERK 41-42 within NAc shell was not affected during the expression session after mGluR5 blockade, suggesting that other proteins are modulating the expression. Furthermore, no study to date has investigated mGluR5 agonists on aspects of cocaine addiction such as conditioned locomotor response. Our hypothesis is that the mGluR5 agonist will promote and enhance the expression of the cocaine conditioned response. Different groups of rats were microinjected with vehicle or CHPG into the NAc shell and placed in the activity chambers during Day 12. No change in the expression of the conditioned response was found.

Abstract 59

ROLE OF OXYTOCIN ON THE ANXIETY ENVIRONMENT-ELICITED COCAINE SEEKING BEHAVIOR AND COCAINE CONDITIONING

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A stress stimulus triggers the reinstatement of cocaine seeking behavior, following long periods of abstinence in dependent subjects. Oxytocin (OT) has been related to reward, learning, memory and stress, events associated with cocaine addiction. Previous data demonstrated an increase in mRNA OT levels within the NAc by acute and chronic cocaine exposure. OT treatments prior to cocaine conditioned stimuli decreased the anxiety levels of the animals, proving that OT is involved in the anxiogenic effects of cocaine actions and might play a modulatory role in the rewarding properties of cocaine. This experiment examines the role of OT on environmentally elicited cocaine-seeking behavior and whether OT reduces anxiety associated with this behavior. OT receptor activates PKC phosphorylate ERK1/2 within HPA. Thus we hypothesized that OT is modulating the stress/anxiety response triggered by environmentally elicited cocaine seeking behavior via MAPK/ERK1/2 within the amygdala and the NAc. Results showed that OT pre-treatment reduced reinstatement of cocaine-seeking behavior. OT treatment reduced the anxiety triggered by cue-induced reinstatement conditions and cocaine-paired conditioned locomotion, demonstrating that OT actions within the brain mediate the anxiety response triggered by cues associated with cocaine intake.

Abstract 60

ORGANIC CHEMICALS AND HEAVY METALS IN URBANIZING WATERSHEDS CAUSE CHANGES ON AGONISTIC BEHAVIORS AND ACTIVITY OF THE PUERTO RICAN FRESHWATER PRAWN MACROBRACHIUM CARCINUS

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Human activities are altering the environment at an alarming rate. Urbanization and the resulting anthropogenic activities have an impact on the quality of water resources and on their fauna. We are thus studying how contaminants found in the island urban rivers affect aggression and general activity of Macrobrachium carcinus. Agonistic behaviors and general locomotion were determined using video recordings before and after exposure to water contaminants. Injection of dibutyl phthalate DBP 0.006 mg/L diethyl phthalate DEP 0.006 mg/L chromium Cr+3 0.100 mg/L and cadmium Cd+2 0.005 mg/L increased the level of activity and altered the locomotor patterns of prawns. Submissive prawns injected with DBP (but not DEP) Cr+3 and Cd+2 increase their aggression levels. Both phthalates and Cd+2 (but not Cr+3) reduce the number and duration of interactions and the number of interactions initiated by the dominant prawns. We are now focusing on determining how the corresponding neural is affected at the circuit, cellular, molecular, and genetic levels.

Abstract 61

VALIDATION OF CANCER-RELATED PROTEINS IN THE HYPOTHALAMIC GT1-7 CELL LINE AFTER EXPOSURE TO ANABOLIC STEROIDS.

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The abuse of anabolic-androgen steroids (AAS) is considered a worldwide health problem. Supraphysiological doses of AAS lead to a variety of neurophysiological problems, including endocrine disorders and cancer-related disease. To determine the biological substrates underlying AAS effects in the neuroendocrine system, we performed proteomic analysis of the hypothalamic GT1-7 cell line after exposure to the AAS, 17α–methyltestosterone. 2D-DIGE and mass spectrometry were used to identify changes in protein expression. We found twelve proteins significatively modulated by AAS. Ontological analysis showed that these proteins were associated with cell cycle/growth, drug detoxification and metabolic processes. Among the up-regulated proteins we found glutathione S-transferase Mu-1 (GSTM1) and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). On the other hand, enhancer of rudimentary homolog (ERH) and phosphatidylethanolamine binding protein-1 (PEBP1) were down-regulated. These results were confirmed by Western blot analysis. Our results highlight the adverse effects of AAS in the neuroendocrine system, specifically in cancer-related processes.

Abstract 62

FUNCTIONAL SELECTIVITY OF THE CANNABINOID RECEPTOR 1 IS REGULATED BY AGONIST-DEPENDENT ENDOCYTIC DWELL TIMES

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Functional selectivity is defined as the process by which a single activated receptor can signal via G proteins or β-arrestin upon distinct ligand binding. The cannabinoid receptor 1 (CB1R) is not only the most abundant receptor in the CNS, but is also a major therapeutic drug target. Despite our understanding that agonist activation of the CB1R induces GRK3 and β-arrestin2-mediated endocytosis, the kinetics of how CB1R signaling is regulated remains unknown

By using total internal reflection fluorescence microscopy, we show agonist-specific dwell times for the CB1R, which is the time that it takes for the receptor to be clustered in clathrin pits with β-arrestin prior to internalization.

Our results show that agonists that induced prolonged dwell times, elicited extended β-arrestin signaling, whereas agonists that induced short dwell times, elicited limited β-arrestin signaling. Furthermore, by preventing CB1R endocytosis we found that β-arrestin signaling could be prolonged.

These findings define a framework by which β-arrestin signaling can be controlled and propose an innovative strategy for the development of therapeutic drugs that target CBRs.

Abstract 63

DUAL BLOCKADE OF FAAH AND TRPV1 WITHIN THE NUCLEUS ACCUMBENS SHELL ELICITS ANXIOLYTIC EFFECTS IN RATS. (2014)

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To our knowledge, the functional role of the co-localization of the endocannabinoid CB1 receptor and the TRPV1 receptor within the mesolimbic system has not been studied. In the present study, we investigated if the co-localization of CB1 and TRPV1 receptors within the NAc shell plays a key role in the emotional responses mediated by the endocanabinoid system, such as anxiety. In the present experiment, male Sprague Dawley rats were implanted with bilateral brain cannulae aimed at the NAcShell. Following recovery from surgery, animals received pre-treatment of microinfusions (0, 0.25nmol/ 0.04µl) of N-arachidonoyl-serotonin (AA-5-HT), a dual blocker of fatty acid amide hydrolase (FAAH) and a TRPV1 antagonist within the NacShell. Following treatment, animals were tested in an elevated plus maze paradigm during 5 minutes. Results showed that pretreatment of AA-5-HT within the NacShell significantly increased open arm entries of animals when compared to controls. The present findings suggest that co-localization of CB1 and TRPV1 receptors within the NacShell may be involved in mediating emotional responses. Future studies with more doses are needed to further characterize these pharmacologic effects.

Abstract 64

METABOLIC CHANGES IN THE MEDIAL PREFRONTAL CORTEX DETECTED BY METABOLOMICS ANALYSIS IN THE MOUSE MODEL OF DEPRESSIVE-LIKE BEHAVIOR

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The role of specific brain regions in the pathophysiology of depression is poorly understood. However, one brain area, the medial prefrontal cortex (mPFC), is emerging as likely being directly involved in major depression. Until now, the molecular mechanisms underlying changes in the mPFC remained unknown. To study metabolic changes in mPFC we applied a mouse model for depressive like behavior described previously by Souza et al, (2013). Samples were analyzed by GC/MS followed by principal component analysis to identify specific metabolites responsible for sample separation. Thus, were detected significant metabolic changes in N-acetyl-aspartic acid, glutamine, pyroglutamic acid, glycine, alanine and serine. In addition, Ingenuity analysis of obtained data sets predicted significant decrease in several biological functions such as, excitation of neurons, long term potentiation, and neurotransmission. Taken together our data suggest that depression causes dysfunction in the gamma-glutamyl cycle- a transport system for amino acids in the mPFC.

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