LSUHealthNewOrleans

NEUROSCIENCE CENTER OF EXCELLENCE

Summer Undergraduate Neuroscience Program

Director of the SUN Program: Nicolas G. Bazan

Coordinator: Brenda Chiappinelli

2014 Summer Research Presentations

Tuesday, July 29th & Wednesday, July 30th

Lions Building, 8th Floor — Room 835

Presentation Schedule

Tuesday– July 29, 2014

10:30 AM – WELCOME
10:45 AM – Kelvin Paul McDaniels
11:00 AM - Emily Tompkins
11:15 AM - Harry Liu
11:30 AM - Sophie Vitter
11:45 AM – Ayanna Banks
12:00 PM – Jordan Anderson
12:15PM – Chelsey Walker
12:30 PM – Clayton Patrick
12:45 PM – Break (15 minutes)
1:00 PM – Lorena Fernandes
1:15 PM – Zavier Davis
1:30 PM - Courtney Chaisson
1:45 PM –Ibrahim Ibrahim
2:00 PM –ADJOURN

Wednesday – July 30, 2014

10:00 AM – WELCOME
10:05AM – Blake Lemoyne
10:20 AM – Kaylyn Martin
10:35 AM – Andres Zabaleta
10:50 AM – Zachary Stielper
11:05 AM – Oluwaseyi Sule
11:20 AM – Break (15 minutes)
11:35 AM – Brad Powers
11:50 AM – Michael Olejniczak
12:05 PM – Nabi Chaudhri-Martinez
12:20 PM – Shela Gu
12:35 PM – Peter Yager
12:50 PM – ADJOURN

Sophie Vitter, Paul McDaniel and Harry Liu and Emily Tompkins
Mentor: Dr. Nicolas G. Bazan

Very Long Chain Polyunsaturated Fatty Acids 32:6 and 34:6 in Preventing Apoptosis

Abstract:

Previous research performed by Dr. Nicolas Bazan on polyunsaturated fatty acids (PUFAs) proved the neuroprotective properties of Docosahexaenoic Acid (DHA), especially combined with Pigment Epithelium-Derived Factor (PEDF). Our research focused on discovering other neuroprotective polyunsaturated fatty acids.

We began by looking at the effects of very long chain polyunsaturated fatty acids (VLCPUFAs) 32:6 and 34:6 on retinal pigmented epithelium cells. We did so by plating both ARPE-19 cells and Human RPE cells and then treating them with these lipids followed by oxidative stress (H_2O_2 and TNF-α). We then compared the amount of cell death in these plates to the amount in untreated control plates.

In addition to 32:6 and 34:6, we also tested the protein Mesencephalic Astrocyte-Derived Neurotrophic Factor (MANF) in the same manner. We compared these results to both control wells and wells plated with DHA, PEDF, and both DHA and PEDF to compare levels of protection and verify our results.

Additionally, we used a 15-Lipoxygenase Inhibitor with the VLCPUFAs to determine if they are following the same pathway as DHA, which requires the 15-LOX enzyme to transform DHA to Neuroprotectin D1. The 15-LOX enzyme was applied to both ARPE-19 and Human RPE cells.

Ayanna S. Banks
Mentor: Dr. Alberto Musto

Characterization of Behavior Stereotypes in a Model of Temporal Lobe Epilepsy

Background: Limbic epilepsy also known as Temporal Lobe Epilepsy, the most common form of epilepsies, is associated with previous brain injury that affects the hippocampus. The time period from injury to spontaneous seizures is denominated "epileptogenesis". Currently, no markers of epileptogenesis have been recognized, therefore it is difficult to predict epilepsy or spontaneous seizures. Our goal is to identify a behavioral marker that could be used as predictor of epileptogenesis. The hypothesis for the project is that there are spontaneous stereotype non-convulsive profiles in limbic epileptogenesis.

Objective: To characterize a novel behavioral phenotype during experimental post status epilepticus-induced epileptogenesis

Methods: The post-status epilepticus (SE) model of TLE induced by pilocarpine (PI) in C57BL/6 adult male mice (20-25g) was used. Mice were placed in individual Plexiglas cages. Spontaneous behavior for samples of five minutes and consecutive five to six hours were recorded using a video recording system. In addition, to evaluate if platelet activating factor (PAF) activity mediates those stereotypes, some mice were treated with PAF receptor antagonist (60 mg/kg PAFr) or vehicle for five consecutive days following SE. Type, number, duration of seizures, Straub tail, and grooming were quantified at different time points after SE by visual analysis.

Results: Preliminary data showed that epileptic mice (2 months after SE, n=4) had an increase of duration and frequency of grooming (23%) and Straub tail (5%) compared to control mice (n=4). In addition, we observed that mice with non-convulsive SE had a trend of increased grooming compared with all convulsive SE treated groups (Vehicle, LAU-09021) except the LAU-09001 treated group, which was higher than the controls. SE Vehicle had a higher increase in the duration of Straub tail than any of the mice; however, non-convulsive mice had the highest frequency of Straub tail. Non-convulsive mice also had a higher number of times it crossed the center of the cage than any of the mice.

Conclusion: According with those observations the Straub tail may indicate signs of non-convulsive epileptic-like behavior as a consequence of SE. The degree of brain damage and/or hyper-excitability could play a role in those behaviors before spontaneous clinical seizure occur.

Jordan E. Anderson
Mentors: Drs. Alberto Musto and Nicolas G. Bazan

Hippocampal Dendritic Spine Organization in Epileptogenesis

Acquired epilepsy is a manifestation of spontaneous, abnormal, electrical brain activity, and clinical seizures as a consequence of previous brain injury. Unfortunately, there are no treatments available to prevent the occurrence of seizures. The re-organization of the neuronal network after brain injury plays a critical role for the development of epilepsy. We observed in hippocampus that dendritic spines, a subcellular component of neurons related with synaptic activity, decrease in number; and some of them show aberrant morphological formations in experimental epilepsy. These changes are associated with micro-epileptiform activities.

The objective of this experiment is to determine the number and lengths of dendritic spines (DS) from dendrites of hippocampal pyramidal and granule cells in an experimental model of epileptogenesis. The post status epilepticus (SE) model of Temporal Lobe Epilepsy, induced by Pilocarpine in C57BL/6 adult male mice (22-27g) was used. Brains were processed following established procedures according to the manufacturer's instructions in the FD Rapid GolgiStain™ Kit, (FD Neurotechnologies, Inc.,Columbia, MD). Coronal sections (80 μm) have been made and then mounted, air-dried, dehydrated in alcohol, cleared in xylene and cover-slipped. Apical and basal dendrites from Golgi-impregnated neurons were selected from the CA1 region using imaging application software (OlyVIA, Olympus, Center Valley, PA). Then dendrites from the Oriens, Radiatum, LM, and Dantate Gyrus (DG) were selected and photographed at 100X. Z stack images (200 frames) were taken with a step size of 0.3 μm using an Axioplan 2 microscope (Carl Zeiss Inc., Thornwood, NY) coupled with AxioCam and Axiovision software (Carl Zeiss Inc.). Dendritic spine density and length of dendritic spines that emerged perpendicular from 10 micron long segments of the dendritic shaft was quantified and recorded using Image J software (National Institutes of Health). The dendrites and dendritic spines were reconstructed into 3D images using Neuron Studios software.

We observed from the Control (N=3; dendrites (nd):8-9; 3 segments of each dendrite) and Epilept`ogenetic (N=2; nd:7-8; 3 segment) mice the following: A) Distribution of DS density show a pattern RAD>OR>DG>LM in controls and RAD > LM> DG> OR in epileptogenesis B) There is a trend of reduction of DS density in all hippocampal area in epileptogenesis. (OR 40% RAD 15% LM 13% DG 19%) showing more impact in OR. In addition: a) Frequency of spines was fewer in mice induced with SE than controls. b) Epileptogenesis tends to cause thick dendrites. c) Dendritic spines show a bow-like shape in epileptogenesis. Preliminary data indicates that epileptogenesis shows a reduction of DS density in the hippocampus, mainly in Stratum Oriens. The dendritic spines in epileptogenesis show a dysmorphic shape. Currently, we are measuring more animals of both conditions (SE and control) in order to have statistical and significant results.

Chelsey P. Walker – LBRN
Mentors: Drs. Alberto Musto and Nicolas Bazan

PAF Antagonist Recovers Dendritic Spines Affected by Epileptogenesis

The development of temporal lobe epilepsy (TLE), also known as limbic epileptogenesis (LE), is a dynamic neuropathological process that occurs during the period between a precipitant injury, such as status epilepticus (SE), stroke, or traumatic brain injury, and the first occurrence of spontaneous seizures. Dendritic spines are essential to post-synaptic activity, and are therefore involved in abnormal neuronal circuitry in epileptogenesis: In epileptogenesis, there is a decrease in dendritic spine density and an increase in aberrant dendritic formations. Previous studies have found that platelet activating factor (PAF), a potent, short-lived phospholipid mediator of inflammation, accumulates in the brain during epileptogenesis. Studies have also shown that PAF receptor antagonism attenuates the loss of dendrites in epileptogenesis and increases spine density and spine length. We aimed to further the body of knowledge about the effects of PAF and PAFr antagonism on dendritic spines by focusing on the effects of PAF antagonism on the distance between dendritic spines and the area of spines on dendrites of pyramidal cells in the CA1 and spines on dendrites of granule cells in the dentate gyrus. We hypothesized that PAF antagonism compensates for the loss of dendritic spines by increasing the size of the dendritic spine neck and head.

In order to better understand the effect of PAF receptor antagonism on dendritic spines during epileptogenesis, we used the the pilocarpine model of epileptogenesis in adult male C57Bl/6 mice. After 24 hours, mice were divided randomly into two groups that were each treated for 5 days with PAFr antagonist LAU-09001 (60 mg/kg, ip) or vehicle (saline, Baxter 0.9%, ip). Brain tissue was collected 7 days after SE. The Rapid Golgi protocol was used. A Zeiss Axioplan microscope was used to perform high objective microscopy (100x). We imaged dendrites in four sections of the hippocampus (statum oriens, stratum radiatum, stratum lacunosum-moleculare, and the dentate gyrus) using the Z-stack method with a step size of 0.3μm. The area of dendritic spines in 10μm sections of

the dendritic shaft along with the gap between adjacent dendritic spines was analyzed using Image J.

Our preliminary results reveal an overall increase in dendritic spine interval (gap) and an increase in spine area in LAU-09001 treated mice compared to control and vehicle mice. This proves that PAF antagonism attenuates the aberrant post synaptic circuitry involved in epileptogenesis by allowing the expansion of surviving dendritic spines. This also shows that the morphology of dendritic spines affected by epileptogenesis may be responsible for abnormal brain oscillatory activity and local field potential.

Clayton Patrick
Mentors: Drs. Alberto Musto and Nicolas G. Bazan

The role of high frequency oscillations in epileptogenesis.

Rationale: Epileptogenesis is a dynamic process involving several molecular and cellular mechanisms that support the rearrangement of neuronal networks which foster the onset of recurrent seizures. High frequency oscillations (HFOs) described in the brain of epileptic animals and patients with epileptic disorders have been postulated as a predictive marker of epileptogenesis. Since HFOs represent hypersynchornized action potentials of small neuronal networks, other abnormal electrical patterns should be studied and characterized to further understand their role in epilepsy. The goal is to simultaneously characterize spontaneous neuronal HFO within different hippocampal regions during epileptogenesis and determine its modulation in the neuronal network activity.

Methods: An experimental model of temporal lobe epilepsy was induced by intraperitoneal administration of kainic acid or pilocarpine in adult mice and rats. Following recovery from status epilepticus (SE), silicone probes with a 16 parallel microelectrode array were implanted in the dorsal hippocampus parallel to the CA1-dentate gyrus axis. Local field potentials from the hippocampus were recorded after being amplified, band-pass filtered (1 Hz-3 kHz) and digitalized with 12 bit resolution at continuous 50 kHz through pre-amplified headstage and system data acquisition systems. Time-dependent changes of the oscillatory activity after SE were analyzed and compared with naïve animals. Analysis included: (1) quantifying bursts of HFOs and (2) frequency band analysis before and after HFO (3) and to determine correlation with brain hyper-excitability.

Results: As expected, HFO events were found in DG regions during sleep-wake transition cycles of animals with clinical spontaneous seizures. HFOs modified the frequency of the subsequent local field potential activity. The frequency analysis of

HFO showed a decrease in delta, theta, and low gamma frequencies after the event in status epilepticus rats, compared to an increase in these frequency bands in control animals. Correlation with seizure episodes showed an increase in theta, beta, and low gamma frequency bands during the preictal period as well as decreased latency and increased number of spikes at seizure onset.

Conclusion: The HFO modulates the neural network, damaging pre-existing neural connectivity leading to a hyperexcitable state. Thus HFO's directly contribute to the hyperexcitable condition of epilepsy and may serve together with responses of neuronal network as a predictive biomarker of epileptogenesis in at risk patients.

Lorena F. Fernandes - Institute of International Education, Brazil
Mentor: Drs. Pranab K. Mukherjee, Nicolas G. Bazan

Docosahexaenoic acid (DHA) and neurotrophins (PEDF and BDNF) modulates Alu-RNA mediated upregulation of NALP3 inflammasome and of pro and anti-apoptotic proteins

An Alu element is a short stretch of DNA originally characterized by the action of the Alu (Arthrobacter luteus) restriction endonuclease. In fact, Alu elements are the most abundant transposable elements in the human genome. It is known that oxidative stress induced by hydrogen peroxide and TNF-α can cause programmed cell death, known as apoptosis, in ARPE-19 cells. DHA in combination with neurotrophin PEDF compromises this apoptosis cell death. Inflammasome is a multiprotein complex that includes a member of Nod-like receptor protein 3 (NALP3) and apoptosis associated speck-like protein (ASC). In my project the influence of Alu-RNA expression was studied in ARPE-19 cells to test the effect of Alu-RNA mediated cytotoxicity and inflammasome associated proteins NALP3, Daxx, and cell survival protein Iduna. Also neurotrophins PEDF and BDNF, in combination with DHA, have been studied on these inflammasome associated proteins in Alu-RNA transfected ARPE-19 cells. Our results indicate that authentic Alu-RNA expression causes cytotoxicity and up regulation of inflammasome proteins, which was compromised by DHA and neurotrophin BDNF in association with DHA. BDNF along with DHA more effectively attenuated Alu-RNA mediated cytotoxicity and protein up regulation compared to PEDF. Our results shed light on the identification of specific cell survival mechanism involving neurotrophin BDNF.

Ibrahim Ibrahim – LBRN
Mentors: Drs. Pranab K. Mukherjee and Nicolas G. Bazan

Importance of Neuroprotective Proteins in Rescuing Brain Samples of Ischemic Stroke Models

Docosahexaenoic acid (DHA) is the most abundant omega-3 fatty acid found in the brain and retina. Preliminary studies show that DHA can slow the progression of Alzheimer's disease by compromising oxidative stress induced apoptosis through upregulating antiapoptotic proteins and down regulating proapoptotic proteins. DHA has been shown to upregulate the neuroprotective protein, Iduna, in both contralateral and epsilateral regions of the brain of Ischemic stroke models. My project was to use a metabolizer of DHA, Neuroprotectin D1 (NPD1), and another analogue, Maresin R1 (MAR1), to detect whether these compounds have better efficacy than DHA itself in upregulating the survival proteins, Iduna and BIRC3, in extracts of Ischemic stroke models induced by middle cerebral artery occlusion (MCAo). The neuroprotective compounds DHA, NPD1, and MAR1 were injected into male Sprague-Dawley rats during MCAo. Saline was used as control during MCAo. Brain samples were collected 1 day, 3 days, and 7 days after introduction of MCAo. Our results indicate that NPD1 was more efficient in inducing the level of expression of Iduna and BIRC3 than DHA as detected by Western Blot Analysis (WB), on the other hand, Maresin 1 displayed much less efficacy than NPD1. Interestingly, the upregulation of BIRC3 was not as prominent as Iduna. This upregulation of Iduna and BIRC3 can be attributed as a protection mechanism in animal models of Ischemic stroke.

Zavier Davis
Mentor: Dr. Ludmila Belayev

Docosahexaenoic acid provides neuroprotection in a chronic experimental model: Characterization by behavior.

Abstract

Background

Docosahexaenoic acid (DHA) is a member of the essential omega-3 fatty acid family that is a prominent neuroprotective effect against experimental stroke. We hypothesized that DHA treatment may improve neurobehavioral deficits after chronic survival following focal cerebral ischemia.

Methods and Results

Male Sprague Dawley rats underwent 2 h of middle cerebral artery occlusion (MCAo) with poly-l-lysine coated filament being removed 2 h after MCAo. DHA (5mg/kg n=9) or saline (0.5% of body weight n=7) was intravenously administered 3 h after MCAo. Neurobehavioral tests (composite neuroscore of postural reflex, visual, tactile and propioceptive placing) on all animals were conducted before the procedure, during MCAo at 1h, 1h after treatment, then on days 1, 2, 3, and weeks 1, 2, 3, and 4. DHA demonstrated the improvement of total neurological score by 27% 1 h after treatment. Neurological score increased by 35-41% at day 1-3 and by 42-45% during weeks 1-4 compared to the saline-treated group.

Conclusions

DHA treatment improves neurobehavioral deficits during 4 week survival following focal cerebral ischemia. Results suggest that DHA endures neuroprotection against chronic ischemic stroke.

Courtney Chaisson – LBRN
Mentor: Dr. Ludmila Belayev

Docosahexaenoic acid provides neuroprotection in chronic survival model of ischemic stroke

Previous studies have shown that docosahexaenoic acid (DHA) has neuroprotective abilities when administered following middle cerebral artery occlusion (MCAo) in a 7 day survival rat model. In this study, we tested whether neuroprotection induced by DHA persist during chronic survival after focal cerebral ischemia. Male Sprague Dawley rats were subjected to 2 hours of MCAo and received intravenously either saline or DHA (5 mg/kg) 3 hours after onset of MCAo. Animals were allowed to survive for 4 weeks and then histopathology was conducted. DHA was shown to significantly reduce the total, cortical, & subcortical infarct area in multiple bregma levels. Furthermore, DHA demonstrated a decrease in the total infarct volume of 68.2% due to a decrease of 71.2% and 54.8% in the cortex and subcortex infarct volumes respectively. This reduction in infarct size serves as an indicator of the efficacy of DHA against chronic cerebral ischemia damage. Therefore, DHA demonstrates the possibility to be applied in clinical settings to treat stroke patients.

Blake Lemoyne – LBRN
Mentors: Drs. Eric Knott and Nicolas G. Bazan

Significance of DHA and 15-LOX-1 in Ischemic Preconditioning: Photoreceptor Survival

Abstract:

Docosahexanoic Acid (DHA) is a 22-carbon long chain polyunsaturated fatty acid that can be converted into the neuroprotective agent, NPD1, with the enzyme 15-lipoxygenase-1 (15-LOX-1). DHA is known to be involved in the protective actions of the brain and retina. To determine how DHA and 15-LOX-1 affect photoreceptor cells during preconditioning, we utilized hydrostatic pressure as well as geared forceps to induce ischemic preconditioning and promote protection in the light damage model of Age-related Macular Degeneration (AMD). Ocular Coherence Tomography (OCT) measurements indicated that hydrostatic ischemic preconditioning provided a higher degree of protection than the geared forceps method. However, after treatment with hydrostatic pressure, many rats developed cataracts while those that experienced preconditioning with geared forceps did not. In light induced photoreceptor degeneration, hydrostatic pressure with controls provided the most photoreceptor protection while light damage without preconditioning offered the least. Addition of DHA and treatment with the 15-LOX-1 inhibitor PD-146176 reduced the protective actions of hydrostatic preconditioning, but exhibited more protection than light damage alone. As DHA and 15-LOX-1 are known to produce NPD1, these findings may help discover the pathway of NPD1 in the retina.

Kaylyn Martin – LBRN
Mentors: Drs. Pranab K. Mukherjee and Nicolas G. Bazan

Modulation of Pro-Inflammatory Proteins and Cell-Survival Proteins by DHA and Neurotrophins (PEDF and BDNF) Under Stress in ARPE19 Cells

Oxidative stress (OS) causes accumulation of Reactive Oxidative Species in ocular diseases such as AMD and Glaucoma. It is already established in this lab that OS using hydrogen peroxide and TNF-alpha cause induction of inflammasome resulting in the accumulation of inflammasome-related proteins like NALP3 and ASC. DHA and PEDF compromise these effects. Contrary to this, Iduna and PIN1, which are two cell survival proteins, behave oppositely with DHA and PEDF. This project will test another neurotrophin, BDNF, in combination with DHA and PEDF on the behavior of above mentioned proteins under oxidative stress. Our results indicate that DHA in combination with PEDF up-regulated Iduna and PIN1 and down-regulated NALP3 in ARPE-19 cells under stress. On the other hand, BDNF, alone or in combination with DHA or PEDF, displayed a comparable effect on those proteins compared to PEDF and DHA. Therefore, BDNF plays an interesting role in the cell survival pathway.

Andres Zabaleta
Mentor: Dr. Ludmila Belayev

DHA Improves Functional Recovery Following Experimental Stroke in Female Rats

Abstract

Previous experiments have demonstrated the neuroprotective effects of DHA after induced experimental ischemic stroke. The purpose of this study was to determine if DHA usage would improve functional ability in female rats after middle cerebral artery occlusion. Female Sprauge-Dawley rats (280-300 g, n=14) underwent two hours of middle cerebral artery occlusion using an intraluminal suture coated with poly-L-lysine while under anesthesia. Two hours after beginning of MCAo the suture was removed and rats were administered either DHA (5mg/kg, n=6) or saline (0.5% body weight, n=8) three hours after onset of MCAo via I.V. Neurological tests were given one hour before MCAo, one hour after MCAo, on days one, two, three, and seven after MCAo. Female rats that were treated with DHA experienced improvements of 33% on day one, 38% on day two, 40% on day three, and 39% on day seven in total neurological scores when compared to rats that were treated with saline. DHA greatly improved neurological deficits in rats that were treated three hours after MCAo in the one week survival model. The neurological improvements demonstrate the potential use of DHA as treatment for recovering female stroke victims. \

Zachary Stielper - LBRN
Mentors: Drs. Pranab K. Mukherjee and Nicolas G. Bazan

Is PAR Binding Necessary for Oxidative Stress-Induced Iduna Upregulation in ARPE-19 cells?

In an attempt to combat neurodegenerative diseases such as Alzheimer's diseases, Parkinson's disease, and Huntington's disease, researchers have sought to identify endogenous molecules that protect cells against harmful stimuli. One such molecule is Iduna, a protein named for the Norse goddess of protection and eternal youth, which has been classified as a poly(ADP-ribose)(PAR)-dependent E3 ligase. Iduna is a neuroprotective protein, as it has been shown to protect against NMDA receptor-mediated glutamate excitotoxicity, ischemia, γ-irradiation, and parthanatos, and it is involved in the recruitment of DNA damage response elements. A study done by Andrabi, S.A., et al. demonstrates that the binding of Iduna to PAR is necessary for Iduna's protection to be elicited. This project focuses on studying the expression of Iduna when treated with other neuroprotective molecules in the context of oxidative stress and whether PAR binding is necessary for docosahexaenoic acid (DHA)-mediated expression regulation. In order to do so, ARPE-19 cells were transfected with either a wild-type Iduna expression vector or a construct containing a mutated version of the protein that is incapable of binding to PAR. Following transfection the cells were challenged with DHA and neurotrophins PEDF and NGF. Western blot analysis was used to quantify expression levels. Our results show a marked increase in the level of wild-type Iduna when treated with DHA, PEDF, NGF, and combinations of DHA and PEDF and DHA and NGF, with little variation in the expressed level of the mutated Iduna form. Taken together, the data suggests that a functional PAR-binding site is necessary for DHA-mediated upregulation of Iduna. Furthermore, nerve growth factor's upregulation of Iduna expression underscores its important role in cell signal transduction.

Oluwaseyi Sule - LBRN
Mentors: Drs. Pranab K Mukherjee, Nicolas G Bazan

Fatty Acid, Amide Hydrolase (FAAH) - URB937 down regulates pro-inflammatory gene COX-2 under stress in Retinal Pigment Epithelial (ARPE-19) cells.

COX-2 is a pro-inflammatory protein which causes harm to body cells when induced by environmental factors or stress which is either induced by ROS (Reactive Oxygen Species) or Interleukins-1β (IL-1β). The current project makes use of two FAAH compounds, ARN2508 and URB937, which are COX-2 inhibitors and should reduce, if not deplete, COX-2 levels in stress-induced ARPE-19 cells. To test this hypothesis, we made two approaches; 1.) To test the compound whether the apoptosis rates induced by oxidative stress (OS) can be compromised by the use of the inhibitors. 2.) To check the COX-2 labels in the ARPE-19 cells induced by either OS or IL1B which induces COX-2 Promoter Luciferase construct in the transfected cells. Western-Blot analysis and Luciferase assay were used to achieve both processes. Our results indicate that the compound, URB937, was able to inhibit oxidative stress induced apoptosis by 33% as compared to FAAH 2508 (43%) at final concentration of 0.5μM. On the other hand, use of higher concentrations (10 μM) of both was toxic to the cells. Interestingly, COX-2 protein induced by OS and COX-2-promoter Luciferase construct in transfected ARPE-19 cells displayed preferential inhibition of Luciferase activity by FAAH compounds. URB937 proved to provide higher activity of COX-2 promoter compared ARN2508. Our results promised therapeutic use of FAAH compound, URB937 in inflammatory diseases.

Brad Powers
Mentors: Drs. William Gordon and Nicolas G. Bazan

Locating ELOVL4 and MFSD2A in Mouse Retina

Abstract:

My project for the S.U.N. Program is locating the elongase ELOVL4 and the transporter MFSD2A in mouse retina and comparing their accumulation between normal mice and mice with degenerating photoreceptors. My hypothesis is that these two molecules will be found in the retinal pigment epithelial layer of the mouse retina and that there will be no difference between the two types of mice. Photoreceptors in the retina, consisting of rods and cones, detect light and transmit visual information to the optic nerve. A large amount of DHA is stored, used, and recycled in the outer segments of the photoreceptors. The elongase ELOVL4 adds carbons to DHA in order to form longer chain fatty acids that aid in the correct functioning of DHA in photoreceptors. Locating ELOVL4 may allow us to know where DHA is elongated in retina and may allow us to locate a potential area of failure for visually deficient patients. The transporter MFSD2A has been found to transport phosphatidylcholine, which contains DHA. Locating MFSD2A allows us to know where DHA is being transported into the retina and eventually into the photoreceptors. MFSD2A and ELOVL4, after extensive testing with immunohistochemistry, were indeed found in the RPE. However, they were also found in the Muller cells of the retina. Also, MFSD2A was found in the photoreceptors of normal mice but not in the knockout mice suggesting that decreased levels of MFSD2A results in photoreceptor degeneration.

Michael Olejniczak
Mentors: Drs. Jorgelina Calandria and Nicolas G. Bazan

"NPD1 regulates cell survival and inflammatory cascade signaling"

Age-related neurodegenerative diseases, including age-related macular degeneration and Alzheimer's disease, have been steadfastly becoming the most pressing medical issues of our time. A hallmark of aged-related macular degeneration and some other retinal degenerative diseases of the eye has been an irregularity in signaling resulting in excessive programmed cell death. Docosahexaenoic acid and its derivative NPD1 are of interest because of the molecules' roles in cell survival and inflammation. A subject of interest in Alzheimer's disease is the role of microglial cells, which function as the resident macrophages of the nervous system. There is evidence that a proponent of Alzheimer's disease involves a repeating cycle of inflammation caused by the activation of microglial cells, which further induces inflammation by endogenous release of neurotoxins by the microglia.

The central hypothesis is that NPD1 regulates a network of genes responsible for cell survival decision making and regulation of inflammatory cascade signaling. Specifically, it increases the expression of antiapoptotic birc3 protein by modulating CREL, the pro-survival subtype of nuclear factor κB. Preliminary results from immunolabeling showed that NPD1 induces cRel translocation into the nucleus. Addition of 200nM NPD1 increases the nuclear content of CREL two fold after 2 hours of oxidative stress stimulation shown by western blotting. Additionally, it is of concern whether NPD1 affects the phenotype of microglial cells by changing from a pro-inflammatory, activated state to a ramified, resting

state. Microglial cells were harvested from rat pup brains, cultured, and then isolated using staining with various primary antibodies. Mass spectrometry will be used to determine the relative abundance of endogenous NPD1 in the cells and medium.

By gaining a greater understanding of CREL translocation by NPD1 and the role of NPD1 in microglial activation, there may be the opportunity to cultivate clinical therapies for degenerative diseases of the eye and brain.

Nabi Chaudhri-Martínez
Mentor: Dra. Haydee E.P. Bazan

Nerve Growth Factor as a possible mechanism of action of PEDF + DHA in Corneal Nerve Regeneration

Abstract

Nerve Growth Factor (NGF) is a neurotrophic factor expressed in corneal epithelium that promotes cell proliferation and wound healing (1). It is responsible for axonal growth and the survival of sensory neurons. Herpes simplex virus-1 (HSV1) infects the majority of the world's population. Ocular HSV-1 infections cause multiple pathologies with perhaps the most destructive being herpes stromal keratitis (HSK), often resulting in corneal scarring and visual impairment. Pigment epithelium derived factor (PEDF) is a multifunctional factor that has neuroprotective and antiangiogenic activities (2,3) and it has been shown that the neuroprotective activity of PEDF for motor neurons is attributable to a 44-mer peptide from the N-terminal of the protein (4), however the mechanism of action is still uncertain. It has previously shown that treatment of corneal epithelial cells with PEDF, in conjunction with the membrane phospholipid-derived docosahexanoic acid (DHA), promotes regenerative corneal innervation. The purpose of my summer studies were a) to investigate if NGF gene expression is increased with PEDF+DHA and/or the 44-mer fragment of PEDF and b) to investigate if treatment of HSV-1 infected rabbit corneas shown differences in inflammation and sensitivity after PEDF+DHA treatment

References:

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Shela Gu
Mentor: Dr. Haydee E.P. Bazan

URB937 and Its Effects on Corneal Healing

Anadamide is a neurotransmitter that acts on endocannabinoid CB1 receptors, which are located on peripheral sensory nerve endings and modulate the perception of pain. Fatty acid amide hydrolase (FAAH) is an enzyme that catabolizes anandamide into arachidonic acid, an ω-6 fatty acid that serves as a precursor for inflammatory prostaglandins. URB937 is a synthetic peripheral FAAH inhibitor that increases anandamide levels and decreases arachidonic acid levels. This project determined if administration of URB937 can promote wound healing in corneal epithelial injuries in mice models.

In the first experiment, 7 week old male mice's corneal epithelium were removed with an Algerbrush II. Either 10 μL of 3 mg/kg URB937 solution or DMSO vehicle were applied topically to the corneas 3 times a day. Mice that were sacrificed at day 1 showed no statistically significant difference in the corneal wound area between URB937 solution and DMSO vehicle (p = 0.487). Mice that were sacrificed at day 2 already had completely closed corneal wounds.

The experiment was repeated again, except the solutions were injected intraperitoneally once a day instead of administered topically. Additionally, platelet activating factor (PAF) was administered topically once a day. This time, URB937 showed statistically significant difference in wound healing compared to vehicle when injected, for both day 1 (p = 0.00138) and day 2 (p = 0.00906). Preliminary immunostaining results showed that URB937 may also decrease neutrophil count compared to vehicle.

Peter Yager
Mentors: Drs. William Gordon and Nicolas G. Bazan

Determining the Lipid Profile of Photoreceptors through LC-ESI tandem triple quad MS/MS

Abstract:

Recently, very long chain poly unsaturated fatty acids (VLC-PUFAs) within the retina have become an area of interest due to their link with an inherited form of juvenile macular degeneration named Stargardt's Disease. Most scientists agree that VLC-PUFAs play an important role in maintaining the structural and functional integrity of the retina, though little is known about their biosynthesis and actual function. These VLC-PUFAs of interest are located on the 1st carbon of the phospholipid, phosphatidylcholine, within the photoreceptors of the retina. Up to this point, a complete lipid profile of photoreceptors has never been determined. However, using very old wild type mice with normal photoreceptors and very old mutant mice with photoreceptor depletion, we hypothesized that we can use mass spectrometry and subtract the lipid profile of mutant mice from the wild types to determine the lipid profile for photoreceptors. By determining the lipid profile, we have located three molecules (54:12, 56:12, and 56:11) that are abundant in wild type mice but minimal in the mutated mice. The presence of these phospholipid molecules establishes a basis for identifying rod photoreceptors within the mass spectra of the total retina, providing a means of monitoring the health and state of degeneration in animal models of human retina disease.

Neuroscience SUN Program 2014
(High school, Undergraduate and Medical Summer Students)

SUN program dates: 5/28-8/1
LBRN program dates: 5/28-7/31 (present in B.R. on 8/1/14)
LSUHSC Student Summer Research Internship: 6/2-7/31
Office of Community and Minority Health Education: 6/3-7/2

	Name	Gender	Program/Affiliation	Interests	Lab Assignment	Co-mentor
1	Jordan Anderson	M	(Senior-Brother Martin H. S.)		A. Musto	------
2	Ayanna S. Banks	F	(Sophomore-Xavier University of LA)		A. Musto	------
3	Kentra Bellard	F	Xavier University)		H. Xia	------
4	Courtney Chaisson	F	**LBRN-** University of Louisiana, Lafayette (Senior-Biology)	Stroke, Epilepsy & Alzheimer's	L. Belayev	N. Bazan
5	Nabi Chaudhri-Martinez	M	University of Puerto Rico Medical Sciences Campus (Freshman medical student)	Ophthalmology	N. Bazan	H. Bazan
6	Zavier Davis	F	Nicholls State University (Senior-English major)	Psychology	L. Belayev	N. Bazan
7	Veaceslav S. Fedorcenco	M	LSUHSC Medical School		H. Xia	------
8	Lorena Fernandes	F	**Institute of Int'l Education-**Xavier University Pharmacy (exchange pharmacy student from Brazil)	Neuroscience research	N. Bazan	P. Mukherjee
9	Sandrine Ferrans	F	Ben Franklin H.S. (Senior)	Science research Goal: MD/PhD	N. Bazan	W. Gordon
10	Shela Gu	F	LSUHSC Medical School (Freshman- medical)	Brain/retinal degeneration	Haydee Bazan	------

11	Ryan M. Hanson	M	**LBRN-** University of New Orleans (Senior-Biology)	Alzheimer's Goal-MD/PhD	N. Bazan	E. Knott
12	Ibrahim Ibrahim	M	**LBRN-** University of New Orleans (Junior-biology/PreMed)	Anesthesiologist	N. Bazan	P. Mukherjee
13	Blake Lemoyne	M	**LBRN-** Northwestern State U, Natchitoches (Sophomore-Bachelor of LA w/ conc. in Scientific Inquiry)	Optometry	N. Bazan	E. Knott
14	Harry Liu	M	**SUN applicant -** Rice University (Sophomore- undeclared)	Retinal degeneration	N. Bazan	W. Gordon
15	Joseph Lockwood	M	LSUHSC Medical (Medical student)	Data analysis	W. Lukiw	----
16	Tracy Mai	F	Xavier University (Sophomore-Biology/Pre-Med)	Eye research	J. Lentz	------
17	Kaylyn Martin	F	**LBRN-**Northwestern State University of LA (Junior-Biology)	Parkinson's (goal-Radiology, Neurology, or Surgery)	N. Bazan	P. Mukherjee
18	Kelvin "Paul" McDaniel Jr.	M	A.T. Still University in MO (Doctor of Osteopathic Med expected May 2015) Masters in Neuroscience May 2011-Tulane B.S. Neuroscience May 2010- Tulane	Ophthalmologist/ Neuroscience research	N. Bazan	LAB- 807
19	Lucas Ochoa	M			J. Lentz	----
20	Michael Olejniczak	M	Tulane University (Junior- Music, Pre-Med)	Medicine	N. Bazan	A. Asatryan
21	Clayton Patrick	M	LSUHSC Medical		A. Musto	--------
22	Keith Perkins	M	(Junior-Southern Univ. New Orleans)		H. Farris	-------
23	Brad Powers	M	University of Notre Dame (Freshman-Pre Med)	Medicine	N. Bazan	W. Gordon
24	Zachary Stielper	M	**LBRN-** Centenary College of Louisiana (Junior-Biology/Neuroscience double major)	Neurological Diseases & Disorders	N. Bazan	P. Mukherjee

25	Oluwaseyi Sule	M	**LBRN-** University of New Orleans **(Freshman- Pre-Med)**	Medicine	N. Bazan	P. Mukherjee
26	Emily Tompkins	F	(first year LSUHSC Medical student)	Ophthalmology-Neuroscience	N. Bazan	LAB-807
27	Sophie Vitter	F	**SUN applicant-** Vanderbilt University (Junior-Neuroscience major, Business Corporate Strategy minor)	Medicine	N. Bazan	W. Gordon
28	Chelsey Walker	F	**LBRN-** Xavier University of LA (Senior-Biology)		N. Bazan	Alberto Musto
29	Ashli Weber		(Sophomore- Xavier University of LA)		X. Li	------
30	Peter Yager	M	(Senior- Saint Paul High School)	Biochemistry /Medicine	N. Bazan	W. Gordon
31	Andres Zabaleta	M	(Senior- Haynes Academy H.S.)	Medicine	L Belayev	N. Bazan
32	Chelsea Burnett	F	**SSP**- Office of Community and Minority Health Education (HS Junior)	Healthcare	M. Jin	-----
33	Isaac Schneiders	M	**SSP**- Office of Community and Minority Health Education (HS Senior-New Orleans Math & Science High Sch)	Medical Technologist	N. Bazan	S. Hong
34	Daeja Shelby	F	**SSP**- Office of Community and Minority Health Education (HS Junior-Lake Area High School)	Biologist	N. Bazan	J. Erickson

Neuroscience Center 2014-SUN Program Schedule

•Wednesday June 4 at 9:30am- 11:45am: NCE large conference room 835- Safety/Ethics training

1:30pm in the DAC: All students, LBRN, SSRP and medical students will attend animal care training with Dr. Leslie Birke

•Beginning on June 5th, every Thursday at Noon: Pizza lunch with Neuroscience Faculty

•Tuesday, June 10th: Students will submit to Dr. Bazan a description of their summer project. This should be in layman's terms and no more than two sentences. Group photo will be taken.

•Wednesday July 2nd: Summer Science Research (4 week HS program) last day in labs

•Monday/Tuesday July 28 & 29th: NCE SUN Program Research Presentations

•Thursday July 31: LBRN and SSRI (Genetics) students last day at the NCE

•Friday August 1st: SUN Program last day/ LBRN poster presentation at LSU in Baton Rouge

Hours and mandatory meetings:

LBRN students are expected to be here 5 days per week and a full day (vacations are not allowed). LBRN mandatory meetings are held every Tuesday from 8:00am-11:30pm.

The **SSR** (4 week HS program) students will be in the labs from 9:00am-3:30pm M-Th. and until noon on Fridays, as they have mandatory Friday sessions at 1:00pm, MEB lecture room B

SSRI (Genetics program) seminars will be every Monday at 3:00 tba by Genetics program

SUN Program: Thursdays- all SUN students- noon pizza luncheon with NCE faculty

Thursday pizza luncheon schedule with NCE faculty:

June 5th: Jeff Erickson

June 12th: Minghao Jin

June 19th: XiaoChing Li

June 26th: Jennifer Lentz

July 3rd: Nicolas G. Bazan

July 7th: Eric Lazartigues

July 10th: Hamilton Farris

July 17th: Haydee E.P. Bazan

July 24th: Chunlai Wu