Eastern Carolina Chapter of the Society for Neuroscience

Presents:

13th Annual Neuroscience Symposium

Catalyst for Collaboration

Featuring the Wooten Lecture by

Mark Mannie, Ph.D.

Professor, Department Microbiology & Immunology
Brody School of Medicine

"Inverse vaccination as a treatment for Multiple Sclerosis"

Focus on Collaboration Session

"Lord of the Dopamine Receptors: Critical Role of D3 in sensory-motor and autonomic function"

[1]Kori L Brewer, Ph.D. and [2]Stefan Clemens, Ph.D.
Departments of Emergency Medicine[1] and Physiology[2]
Brody School of Medicine
East Carolina University

Tuesday, November 1st, 2011
East Carolina Heart Institute
8:30 am - Registration
9:00 am - Program Begins

Registration and Abstract Submission information at
www.ecu.edu/neurochapter/

The Officers and Council Members of the Eastern Carolina Chapter of the Society for Neuroscience would like to express their sincere gratitude to the following entities for their support of the 2011 Annual Neuroscience Symposium:

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2011 Eastern Carolina Chapter of the Society for Neuroscience
Annual Neuroscience Symposium

Schedule of Events:

8:45-9:15 Registration

9:00-9:15 Opening Remarks: Dr. Sherri Jones and Dr. Dierdre Mageean

9:15-9:30 **Amy Friesland**, Dept. of Anatomy and Cell Biology
"*ZCL278, a Small Molecule Targeting Cdc42 Subclass in Rho GTPase-Mediated Neuronal Morphogenesis*"

9:30-9:50 **Dr. Liz Dugan**, Dept. of Emergency Medicine
"*Analgesic benefits of active exercise for incomplete spinal cord injury associated neuropathic pain*"

9:50-10:05 **Marcoita Gilbert**, Dept. of Pharmacology
"*CB_1 cannabinoid receptor activation reduces auditory habituation and disrupts related Arc/Arg3.1(Arc) expression in auditory regions of zebra finch telencephalon*"

10:05-10:25 **Dr. Jian Ding**, Dept. of Physiology
"*Circadian Neurobiology and Malaria*"

10:30-10:45 Break/Vendors

10:45-11:30 **Focus Session: "Lord of the Dopamine receptors: critical roles of D3 in sensory-motor and autonomic function"**

Dr. Kori Brewer, Dept. of Emergency Medicine
"*D3 receptor dysfunction alters baseline sensory function and morphine responsiveness in the spinal cord*"

Dr. Stefan Clemens, Dept. of Physiology
"The Dopamine D3 receptor knockout mouse mimics aging-related changes in autonomic function and heart"

11:30-1:30 Posters/Vendors/Lunch (presenters have assigned hour to be at poster)

1:30-2:00 **Dr. Michael Rotondo**: **ECU Center for Clinical Neuroscience Update:**
(Introduction by Dr. Paul Cunningham)

2:00-3:00 **Wooten Lecture**: **Dr. Mark Mannie** (Introduction by Dr. Bob Lust):
"*Inverse vaccination as a treatment for Multiple Sclerosis*"

3:00 Closing Remarks and Awards

ZCL278, a Small Molecule Targeting Cdc42 Subclass in Rho GTPase-Mediated Neuronal Morphogenesis

Amy Friesland and Qun Lu

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Neuronal branching and synaptic density play important roles in brain activity. Inadequate arborization is one of the fundamental defects in mental retardation and neurodegenerative diseases. As a key regulator of growth cone morphogenesis, Cdc42 is not only crucial for many cellular functions including membrane trafficking, cell cycle control, and cytoskeletal organization but also for brain development, as conditional Cdc42 knock-out mice die at birth and show gross brain abnormality In humans, aberrant Cdc42 signaling contributes to both mental retardation and Aarskog-Scott Syndrome, a genetic condition associated with mental retardation as well as facial and skeletal abnormality. Unlike Rho GTPase family members RhoA and Rac1, a more complete understanding of Cdc42 has lagged behind due to the absence of specific small–molecule modulators. We have therefore employed computer-assisted virtual high-throughput in silico screening and identified compounds that were able to fit into the surface groove of Cdc42 that is critical for guanine nucleotide exchange factor (GEF) and activation of the GTPase. We demonstrate by imaging methods and biochemical assays that ZCL278 is a selective small-molecule that can inhibit the formation of the GTP-bound (active) Cdc42. ZCL278 disrupts Cdc42-mediated microspike formation and GM130-docked Golgi structures. Additionally, ZCL278 reduces peri-nuclear accumulation of active Cdc42, which could not be accomplished by a specific Rac1 inhibitor, NSC23766. Using ZCL278 as a tool, we confirm the importance of Cdc42 function to both growth cone dynamics and neuronal branching. Therefore, through the identification of ZCL278, we have now gained a powerful tool for further elucidating the functions of Cdc42 in human diseases. This study was supported in part by NIH NCI-CA111891 and the Wooten Laboratory for Alzheimer's and Neurodegenerative Diseases.

Analgesic Benefits of an Active Exercise Protocol for Neuropathic Pain Symptoms in Adult Rats.

Elizabeth A. Dugan, Brian Whitfield, Kori L. Brewer

Department of Emergency Medicine, Brody School of Medicine at East Carolina University

Damage to the dorsal grey matter of the spinal cord produces pathological changes that result in neuropathic pain symptoms. Currently, neuropathic pain is primarily treated with pharmacotherapy which is widely ineffective. Recent clinical reports suggest that spinal cord injury patients undergoing physical therapy to treat locomotor deficits also report relief of neuropathic pain symptoms. Neither the mechanisms nor the degree of physical therapy needed to produce these analgesic effects are known. We examined the effects of an intense active treadmill exercise for the prevention and treatment of neuropathic pain symptoms following excitotoxic dorsal horn damage. METHODS: Adult male Long-Evans rats received a unilateral, intramedullary injection of quisqualic acid (QUIS) into the superficial grey matter of the T_{11}-T_{12} dorsal horn, an injury model that reliably produces central pain syndromes including below-level hyperalgesia and at-level over grooming behavior. Animals were assessed for the development of hyperalgesia using latency withdrawal times to a thermal stimulation (Hargreaves), and monitored for the presence of over grooming behavior. The day that either pain symptom emerged, the animals we placed on an intense (20m/min 8° incline) active treadmill protocol consisting of 5 days/week for two 30-minute sessions with a 10 minute break between sessions. Hargreaves analysis was conducted weekly on Mondays before active treadmill exercise and on Fridays after the last exercise session. Over grooming behavior was scored daily and photographically documented weekly. Treadmill exercise continued for 12 weeks at which time the animals were sacrificed. The thoracic-11/12 lesion and lumbar enlargement (L4-5, associated with the hindlimbs) of the spinal cord were harvested for analysis of inflammatory markers, endogenous opioids and associated receptors and neurotrophic factors. RESULTS: Behavioral analysis showed that intensive active treadmill exercise provided analgesic benefits to animals that had developed over grooming behavior and hyperalgesia. Over grooming behavior did not increase in severity compared to non exercised controls however, the area that the over grooming encompassed continued to increase. Withdrawal latencies to a thermal stimulus increased above baseline latencies, suggesting that active treadmill protocol provided analgesic benefits that did not diminish over time. Thermal withdrawal latencies differed between Friday and Monday sessions. Withdrawal latencies immediately after the 5^{th} day of active exercise where lower than those observed after 2 days off of active exercise suggesting that the analgesic benefits are persistent over time even though there appears to be an immediate hypersensitivity, possibly due to a wind up effect of the sensory system. CONCLUSIONS: Intense, forced exercise reduced the hypersensitivity to thermal stimuli and slowed the progression of over grooming behavior in rats following destruction of the dorsal horn. Behavioral results need to be correlated to anatomical and molecular outcomes to determine the possible mechanism behind this effect.

CB_1 cannabinoid receptor activation reduces auditory habituation and disrupts related Arc/Arg3.1(Arc) expression in auditory regions of zebra finch telencephalon

Marcoita T. Gilbert, Ken Soderstrom

Department of Pharmacology and Toxicology, Brody School of Medicine, East Carolina University, Greenville NC 27834 USA

Cannabinoid agonists produce an array of cognitive impairments; however and understand of the physiological mechanisms responsible remains incomplete. We have found that chronic exposure to cannabinoids alters dendritic morphology and interferes with zebra finch song learning. We are currently working to understand mechanisms responsible. Arc, an immediate-early gene product necessary for long-term potentiation, is rapidly induced in zebra finch NCM (a secondary, integrative auditory region of telencephalon) in response to hearing novel song, and gradually habituates after repeated presentations of the same song stimulus. Arc's mRNA/protein temporal expression parallels other immediate early genes, but is unique in that its mRNA rapidly distributes throughout the dendrite and localizes to areas that have received direct synaptic input. Distinct dendritic Arc expression suggests a potential role in cannabinoid-altered dendritic morphology. We propose that altered vocal learning may involve a CB_1 activity-related disruption of Arc protein expression. To this end, a series of experiments were conducted in order to test the ability of the cannabinoid agonist WIN55,212-2 (WIN) to acutely disrupt song-induced Arc expression, interfere with recognition of song, and alter dendritic spine densities associated with learning. One-way ANOVA revealed that acute WIN treatment (3 mg/kg) significantly reduced Arc expression within NCM. In addition, this molecular correlate of song recognition was blocked by chronic treatment with WIN, and reversed with the CB_1-selective antagonist SR141716A (6 mg/kg). WIN differentially influences Arc expression in thalamic versus telencephalic auditory brain regions, suggesting that Arc is involved in telencephalic but not thalamic CB_1-initiated signal transduction. In addition, WIN pretreatment prevented acute effects of novel song exposure to increase dendritic spine densities in secondary auditory telencephalon (NCM), suggesting a relationship between cannabinoid-reduced Arc expression, inappropriate spine morphology, and an inability to encode/consolidate auditory information properly during learning. In summary, WIN-induced inhibition of Arc expression in auditory NCM may explain cannabinoid-altered vocal development and related persistently-elevated dendritic spine densities. Supported by NIDA R01DA020109.

D3 receptor dysfunction alters baseline sensory function and morphine responsiveness in the spinal cord

[1]Kori L. Brewer, Ph.D.,[1]Christine Baran and [2]Stefan Clemens, Ph.D.

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Activation of the mesolimbic dopamine (DA) system plays an important role in modulating spinal cord function, including the regulation of pain sensitivity, a role primarily attributed to the opioid system. Despite a growing awareness of the combined role of DA and opioids on pain regulation, few studies have attempted to decipher the functional consequences of DA and opioid-related interactions in the spinal cord. In a clinical model of chronic pain, Restless Legs Syndrome (RLS), both DA D3 receptor agonists and opioids provide beneficial effects on the abnormal pain sensation, but only D3 agonists do not lead to sensitization. Previous *in vitro* studies have shown that manipulating DA receptors in spinal circuits results in altered reflex properties that were primarily via D3 receptor pathways, and that the responsiveness of DA receptors can be changed by application of exogenous opiates. Here we provide now the first evidence of the interactions of DA and opioids in an animal model of RLS, the D3 receptor knockout mouse (D3KO). Our preliminary data indicate that mice lacking the D3 receptor have lower pain thresholds, altered response to morphine, and altered endogenous opioid system when compared to wild-type controls.
Thus this study opens new avenues to define the role of D3 receptors in pain processing and their interactions with the well-established opioid system in the RLS model, and it may also represent a new model of chronic pain that is applicable to other than RLS patients.

The Dopamine D3 receptor knockout mouse mimics aging-related changes in autonomic function and heart architecture

Stefan Clemens, PhD, Benjamin E. Keeler, PhD, Tracy Johnson, Christopher Jones, Dave Tulis, PhD, Jitka Virag, PhD, Robert M. Lust, PhD

Department of Physiology, Brody School of Medicine, East Carolina University

Aging is a complex composite of several different processes that can be distinguished from age-related diseases (e.g. Alzheimer's, Parkinson's), and with age, there is an increased risk of diabetes, cardiovascular, and hypertension. Intriguingly, there is ample evidence for a role of dopamine (DA), and the D3 receptor in particular, in the pathophysiology of hypertension. Moreover, patients suffering from the sensory-motor and sleep disorder Restless Legs Syndrome (RLS), a disorder that is primarily treated with dopamine D3 receptor agonists, have a high incidence of increased autonomic output pointing to a possible common abnormality in RLS and during aging. Therefore, we here asked the question if the aging-related changes in autonomic output and corresponding cardiac function observed in wild-type (WT) animals could also be found in young D3 receptor knockout (D3KO) animals, possibly indicating signs of advanced cardiac aging in the knockouts.
Using non-invasive blood pressure measurements, functional echocardiography, and immunohistochemistry of the hearts of differently-aged WT animals (2-3 months, 1 year, 2 years) we found an aging-related increase in blood pressure and cardiac functions, which was accompanied by bradycardia in the oldest animals. Intriguingly, the 2-3 month old D3KO cohort displayed blood pressure and heart rate values that were significantly increased over their age-matched WT controls, and similar to those of the 2 years-old WT group. Further, while the echocardiography did not reveal significant differences between young WT and young D3KO, the immunohistological analyses revealed an aging-related increase of fibrosis in the heart of WT animals, with the fibrosis of the oldest WT animal group being similar to the one observed in the young D3KO. Taken together, our data suggest that the D3KO animal may also serve as a model to better understand the role of the dopamine D3 receptor in the aging heart, and in particular its role in the pathophysiology of fibrosis.

The Wooten Lecture:

Inverse vaccination as a treatment for Multiple Sclerosis

Mark Mannie, Ph.D.

Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University

Multiple Sclerosis (MS) is an inflammatory neurodegenerative disease that afflicts approximately 350,000 Americans and over 2 million persons in the western world. MS is typically associated with an overt inflammatory response against CNS myelin that gradually evolves into a progressive neurodegenerative disease. Clinical research has now shown that broad-spectrum anti-inflammatory strategies are effective in delaying the progression of MS. However, no cure exists for MS, and broad-spectrum immunosuppression is associated with severe adverse side effects. Inverse vaccination is an emerging alternative approach for therapy of MS. Inverse vaccination, unlike classical vaccination, drives an antigen-specific subset of immunosuppressive T cells that elicit a highly specific immunosuppression with an enduring immunological memory. This approach is designed to 'turn off' only those lymphocyte clones responsible for disease without affecting the vast majority of other lymphocyte clones that provide adaptive immunity. This presentation will focus on our laboratory's effort to develop a unique class of inverse vaccines to prevent and reverse MS-like disease in rodents. The concept of inverse vaccination may be relevant to many neurodegenerative diseases, given the emerging awareness that inflammatory reactions are associated with the etiology and progression of neurodegeneration in Alzheimer's and Parkinson's Disease.

Expression of Postsynaptic Scaffolding Proteins and Development Changes in the Inner Hair Cell-Spiral Ganglion Cell Synapses

Jeremy P. Braude and Sonja J. Pyott

UNC Wilmington

The inner hair cell-spiral ganglion cell synapse is the first site of chemical neurotransmission in the sensation of auditory stimuli. This synapse is glutamatergic and undergoes morphological and physiological changes during the maturation of hearing. Using immunofluorescence and confocal microscopy, we examined the expression of postsynaptic scaffolding proteins associated with central glutamatergic synapses in excised mouse organs of Corti. We also quantified the number of presynaptic ribbon and postsynaptic densities in excised organs of Corti from mice aged postnatal day 6 (before the onset of hearing) through 21 (after the maturation of hearing). We observed expression of most postsynaptic scaffolding proteins at the inner hair cell-spiral ganglion cell synapse and extensive pruning of both presynaptic ribbons and postsynaptic densities during hearing maturation. These findings are suggestive of activity-dependent stabilization of postsynaptic sites during hearing maturation. However, it remains unclear what drives pruning of the presynaptic ribbon terminals.

The Effects of Paternal High Fat (HF) Diet on Neurobehavioral Function in Mice

Iola D. Conchar[1], Lily Medina[1], Dorothy Dobbins[3], Elena Pak[4], Alexander Murashov[4] and Tuan D. Tran[1,2]

[1]Department of Psychology, East Carolina University, Greenville, NC; [2]Multidisciplinary Studies Program in Neuroscience, ECU; [3]Interdisciplinary Studies Program in Biological Sciences, ECU; [4]Department of Physiology, ECU Brody School of Medicine

Obesity is an important global health problem and a risk factor for morbidity and mortality. Many studies have examined the maternal contributions of transgenerational effects, showing that epigenetic mechanisms of a high-fat diet may negatively impact glucose regulation and neurobehavioral/cognitive function in offspring (Greenwood et al., 1996, 2005, 2008). Interestingly, some evidence shows that obese and diabetic fathers are more likely to have obese offspring predisposed to diabetes (Harjutsalo et al., 2006; Loombda et al., 2008) and more recently, it has been shown that the offspring of obese fathers have higher body fat composition and lower glucose tolerance (Parker et al., 2010), further bolstering the notion that nongenetic transgenerational effects may be contributing to enduring physical and metabolic deficits in affected offspring. Yet there is paucity of information about whether paternal contributions may also underlie some of the neurobehavioral deficits in offspring. The present study examined the effect of paternal high-fat (HF) diet on susceptibility to brain and cognitive deficits in adult offspring. Adult male C57BL/6J mice were placed on a diet regimen for 12 weeks: (1) 60% fat chow (high-fat, HF); (2) 10% fat chow (control); or (3) an motor exercise group on 10% fat chow. The males were mated with normal C57BL/6J females and their adult offspring were examined for changes in physical, glucose, and neurobehavioral measures. Each mouse will undergo two well-studied learning paradigms: (1) spatial learning in the Morris water maze and (2) trace eyeblink classical conditioning (TECC), both of which assess hippocampal function. Afterwards, their hippocampi will be examined for cell loss in key populations essential for spatial learning and TECC. Data generated from this study may elucidate transgenerational effects of HF diet on offpring susceptibility to neural and cognitive deficits. This research was supported by ECUDOI Pilot Grant to AKM and internal funds to TDT.

Spinal Cord Function and Dopaminergic Modulation in a Mouse Model of Alzheimer's Disease

Sebastian DeMarco and Stefan Clemens, PhD

Department of Physiology, Brody School of Medicine, East Carolina University

The pathophysiology of Alzheimer's disease (AD) as a neurodegenerative disorder has been well characterized in the brain, but the disease's effects in the spinal cord are largely unknown. Several recent studies suggest that AD causes disease-related changes in both brain and spinal cord, but the functional consequences of AD in the cord have not been researched. Our study aims to reveal the differences between spinal reflex amplitudes of wild type (WT) control animals and a triple transgenic mouse model of Alzheimer's disease (3xTg-AD) and their modulation by dopamine (DA). We isolated young (P5-P15) mouse spinal cords in vitro and bathed them in artificial cerebrospinal fluid. After attaching suction electrodes on dorsal and ventral nerve roots of lumbar spinal segments, dorsal roots were electrically stimulated with sufficient amplitude and duration (100-500 μA, 50-500 μs) to recruit sensory nerve fibers so that reflex responses could be recorded at the ventral roots. Dopaminergic drugs SKF 38393 (an excitatory D1 receptor agonist) and quinpirole (an inhibitory D2 receptor agonist) were bath-applied, and the reflexes measured were compared to the corresponding pre-drug conditions between the animal strains. We hypothesized there will be a difference in spinal reflex function between the WT and 3xTg-AD model, because aging in healthy mice is accompanied by a reconfiguration of DA receptor distributions in the spinal cord, and AD may also cause changes in DA receptor distribution. We found that stimulus intervals of 30 s or 60 s consistently led to a gradual decline in spinal reflex amplitudes in 3xTg-AD mice, but not in WT animals. Furthermore, our results show a difference in reflex function between the WT and 3xTg-AD mice. For monosynaptic reflexes (MSRs), WT animals showed a consistent increase in reflex amplitude (average 50% increase, $p<0.001$) with the application of SKF 38393, and a consistent decrease in reflex amplitude (average 25% decrease, $p<0.001$) with the application of quinpirole. In contrast, individual experiments on the 3xTg-AD animals showed either increases or decreases in MSR amplitude. Overall effects for both drugs on 3xTg-AD were not significant (SKF: average 34% increase, $p<0.354$; quinpirole: average 7% decrease, $p<0.888$). For longer latency reflexes (LLRs), WT animals showed a consistent increase in reflex amplitude (average 105% increase, $p<0.001$) during SKF application, but no statistically significant effect with quinpirole (average 13% increase, $p<0.618$). As with the WT mice, LLRs in 3xTg-AD animals showed a consistent increase in reflex amplitude with SKF (average 235% increase, $p<0.001$), but statistically insignificant effects for quinpirole (average 18% increase, $p<0.464$). These preliminary data suggest that the neural circuits in the spinal cord of 3xTg-AD animals have a modified responsiveness to DA. This points to a pathophysiological role of Alzheimer's disease on spinal cord function, and future studies with this transgenic mouse model will reveal more information about these changes. Supported by the Harriett and John Wooten Foundation for Alzheimer's and Neurodegenerative Disease Research.

Assessment of Neurobehavioral Outcomes using a Triple-Transgenic Mouse Model of Alzheimer's Disease

Dorothy L. Dobbins[1], Iola D. Conchar[2], Lily M. Medina[2], Mark Mannie[3], Qun Lu[4] and Tuan D. Tran[3,5]

[1]Department of Biology, ECU; [2]Department of Psychology, ECU; [3]Microbiology, BSOM; [4]Anatomy and Cell Biology, BSOM; [5]Multidiscplinary Studies Program in Neuroscience, ECU

Alzheimer's disease (AD) is characterized by severe cognitive dysfunctions, including memory loss and difficulty with spatial awareness, severely hindering everyday performance for those it affects. AD is the primary cause of dementia and contributes to 60-70% of cases (Barker et al., 2002). About 5.3 million Americans (roughly 12.5%) over 65 are afflicted with AD (Holtzman et al., 2011). Although AD generally plagues the elderly, brain degeneration and cognitive dysfunction can occur 10-20 years before dementia onset. An alarming number of individuals are affected by AD worldwide (~30 million) and by extension, the burden of this disease is encumbered by patients' families, caregivers, and society at large, prompting tremendous efforts by researchers and clinicians to translate their findings into developing efficacious therapies. AD is thought to be a disorder involving multiple genetic abnormalities and cellular pathways (Holtzman et al., 2011), and current studies using genetic methods may lead to new insights into its pathogenesis. Indeed, present studies using triple-transgenic (3xTg-AD) mice expressing APP-Swe, PS1-M146V and tauP301L mutations are helping to this end. In this study, we will examine whether 3xTg-AD mice exhibit neurobehavioral deficits across the lifespan, thus modeling disease progression in humans. At 3, 5, and 7-months of age C57BL/6J and 3xTg-AD mice will be exposed to 10 days of water escape training using the place version of the Morris maze (7 acquisition; 3 probe). Afterwards, they will be trained to the "trace" version of eyeblink classical conditioning (ECC) for 6 consecutive days. Learning in both tasks is mediated by an intact hippocampus, a primary target of AD pathology. Because the 3xTg-AD mice bear all three hallmark features of AD seen in humans, we hypothesized that they will exhibit impairments in acquiring both tasks successfully, particularly as they age. After ECC is complete, their hippocampi will be examined for cell loss in key populations that interact to mediate learning and memory processes. It is hoped that data gathered from these assessments will provide greater insight on the differential brain deficits and cognitive impairments resulting from AD, thus opening possibilities for developing experimental therapeutics that minimize its pathogenesis.
Funding: Wooten Foundation grant to MM.

A Role for GSK-3 in the Treatment of Spinal Cord Injury Pain

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Spinal cord injury (SCI) is a serious clinical condition that in most people results in chronic pain and dysesthesias. Neuropathic SCI pain is partly a result of abnormal neuroplasticity characterized by sensory neuron sprouting. Glycogen synthase kinase 3 beta (GSK-3β), an intracellular mediator of neuronal growth and survival, represents a potential neurochemical target for the treatment of spinal cord injury pain. The purpose of this study was to investigate the pharmaceutical modulation of GSK-3β activity for its ability to prevent sensory neuron sprouting after SCI. Male, Long-Evans rats underwent excitotoxic SCI through intramedullary injection of quisqualic acid (QUIS; n=6) or an equal volume of saline for controls (n=8), into the dorsal gray matter. Animals were examined daily for overgrooming behavior. After 14 days, spinal cords and dorsal root ganglia (DRG) were removed from the level of injury. DRG neurons ipsilateral to the site of injury were cultured and treated with GSK-3β activators (LY-294002) or inhibitors (LiCl or SB415286). Eighteen hours after plating neurons were analyzed for sprouting and overall neurite length. SC injured (QUIS-injected) animals demonstrated a significant ($p < 0.05$) increase in neurite length (61.6 ±10.5 μm) compared to saline-injected control animals (22.9 ±2.7 μm). Drug treatments on cultured DRG from saline-injected animals showed that GSK-3β inhibitors (LiCl and SB415286) increased neurite lengths (LiCl 44.4 ±12.4μm, SB 48.8 ± 11.1μm).SC injured (QUIS-injected) animals demonstrated a significant ($p < 0.01$) increase in DRG neuronal sprouting (46.2%) compared to saline-injected controls (30.5%). Application of GSK-3β (LY-294002) activator on DRG from QUIS injected animals resulted in a significant ($p < 0.0001$) decrease in the extent of sprouting in QUIS animals (21.7%), reducing the percent of sprouting below saline-injected controls levels. Our results show that excitotoxic SCI resulted in DRG sensory neuron sprouting that could be correlated with pain behaviors, and that *in vitro* pharmaceutical activation of GSK-3β reduced DRG sprouting to non-injured levels. This study shows that pharmaceutical activation of GSK-3β influences sensory neuron sprouting, implicating GSK-3β as a novel therapeutic target for SCI pain. Funding provided by the Wooten Foundation and ECU's Department of Research and Graduate Studies.

Development of Non-Peptidyl Lysosomal Modulators for the Treatment of Frontotemporal Dementia

Rebecca A. Howell[1], Meagan LWisniewski[1], Dennis J Hoover[2], Yuliya Sumskaya[2], Jeanie Hwang[1], Kishore Viswanathan[3], Dennis Wright[3], and Ben A. Bahr[1,2].

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Lysosomes are the primary site for removal of old and misfolded proteins which maintain cellular homeostasis. Positive lysosomal modulation has been shown to enhance protein clearance to protect against protein accumulation pathology. Small-molecule lysosomal modulators, such as Z-Phe-Ala-diazomethylketone (PADK), at appropriate concentrations elicit marked up-regulation of cathepsins and other lysosomal enzymes without any indications of synaptopathogenesis, behavioral abnormalities, or organ malfunctions. Recent work designed and synthesized compounds to improve potency, selectivity, and metabolic stability. We utilized the PADK structure as a departure point to develop a non-peptidyl lead series that removed the internal amide linkage, eliminated the highly reactive diazoketone moiety, and developed highly flexible, enantioselective routes for first-in-class compounds. The lead series maintained or improved the modulatory profile of PADK, providing protection in the hippocampal slice model of protein accumulation that exhibits experimentally-induced phosphorylated tau deposits. We are currently using Taconic's transgenic mouse line 2508 that expresses the P301L mutation in human tau that causes the disease. Brain slice cultures from the transgenic pups will be tested for vulnerability to chloroquine induced tau accumulation as well as lysosomal modulator testing to detect reduction in protein. The Taconic 2508 mother at 6 months old will be tested for expressed paired helical filaments (PHFs) and associated synaptic decline as well as lysosomal modulation for protection of synaptic markers and reduction of paired helical filaments. The promising compounds will be used to further design efficacious modulators, for protective agents that enhance protein clearance, promote synaptic integrity, and slow the progression of proteinopathies.

Segment-Specific Dopamine Meta-Modulation of the Monosynaptic Stretch Reflex in the Spinal Cord

Tracy Johnson, Stefan Clemens

Brody School of Medicine, Department of Physiology, East Carolina University

Descending dopamine (DA) fibers project throughout the entire length of the spinal cord, but DA's modulatory actions on sensory-motor function have generally only been assessed in the lumbar segments L2-L5, which provide the output for the central pattern generator for locomotion. In contrast to L2-L5, spinal cord segments T1-L2 and, in mouse, L6-S2, also contain intermediolateral nuclei (IML), which house the final common output of the autonomic nervous system (ANS). Here, we provide evidence that DA modulation of the monosynaptic reflex (MSR) circuitry has opposing effects on reflex amplitude in ANS-containing segments when compared to L2-L5 segments.

Spinal cords of male and female mice (C57BL/6, postnatal days 5-14) were isolated in aerated (95%O2/5%CO2) artificial cerebral-spinal solution, and pairs of suction electrodes were attached at dorsal and matching ventral roots of thoracic, lumbar, or sacral segments. Dorsal roots were stimulated with constant-current pulses (200-500 μA, 100-500 μs, intervals of 30-60 s), and the reflex responses at the ventral roots were recorded, amplified, and digitized for later analysis (pClamp).

As shown previously, application of low DA (1-5 μM) led to a decrease in MSR amplitude in L2-L5 segments ($n=4$, $p<0.002$). However, in the ANS-containing segments low DA facilitated the MSR response ($n=4$, $p<0.001$). We did not observe any difference between thoracic (sympathetic) and lumbosacral (parasympathetic) segments. Further, application of the D1-receptor agonist SKF 38393 facilitated the MSR in L2-L5 ($n=4$, $p<0.001$), but decreased it or had no effect in SNS or PNS-containing segments ($n=2$, $p=0.4$). The D2-receptor agonist bromocriptine (10 μM, $n=6$) decreased the MSR in L2-L5 segments ($p<0.001$) but increased it in ANS segments, whereas blocking D2 receptor pathways with raclopride (10 μM) had opposite effects, leading to an increase of the MSR in L2-L5 segments ($n=7$, $p<0.001$) and a concomitant decrease of the MSR in ANS-segments ($n=9$, $p<0.001$).

Gap junctions have been reported between IML and motoneuron populations, suggesting a possible mechanism for MSR modulation by the ANS. Therefore, we tested the effect of the gap-junction blocker, carbenoloxone, in conjunction with the dopaminergics. We found that the addition of carbenoloxone (100 μM) to the DA drugs reversed the MSR response observed in the ANS segments, such that it became similar to the response in L2-L5 segments ($n=4$).

Our data suggest that the outcome of DA modulation on the spinal MSR circuitry is dependent on the presence or absence of the ANS, and that gap junctions between IML and motoneurons may play an important role in this meta-modulation.

Differential Frequency-Dependent Plasticity of Monosynaptic and Longer Latency Reflexes in the Spinal Cord is Dopamine D3 Receptor- and Calcium Channel-Dependent

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Frequency-dependent plasticity (FDP) of the monosynaptic stretch reflex (MSR) has been well explored in the spinal cord, but there exists no characterization of the modulatory effects of FDP on the C-fiber associated longer-latency reflexes (LLRs). C-fibers terminate in the superficial dorsal horn, and neurons in Lamina I are one of the main targets of descending dopamine (DA) fibers from the dorsal hypothalamus. As spinal D3 receptors are involved in MSR modulation, we examined the role of FDP on LLRs and its interactions with DA signaling in wild-type mice (WT) and DA D3 receptor knockout mice (D3KO), an animal model for increased spinal sensory sensitivity. Using isolated in vitro spinal cord preparations (P7-14), we applied constant current pulses (200-500 μA, 100-250 μs, 0.5-20 Hz) to dorsal roots of the lumbar cord. Reflex responses were recorded at the corresponding ventral roots, grouped as MSRs or LLRs, and compared to the 1st response in a 5-pulse stimulus train. At the end of the stimulus train, WT MSR response decreased to 45.2±10.5% at 0.5 Hz, and to 27.4±2.9% at 20 Hz. D3KO MSR responses at 0.5 Hz decreased to a similar extent as in WT (to 44.4±20.0%), but decreased below WT levels at 20 Hz, to 13.3±3.5% ($p<0.001$), suggesting an increased sensitivity of the D3KO MSR response to FDP at higher frequencies. Similar to the MSR, LLR responses in WT decreased to 33.5±17.4% at 0.5 Hz and to 20.3±5.6% at 20 Hz. In contrast, in D3KO, LLR responses at 0.5 Hz decreased less than observed in WT (to 72.8±12.5%, $p<0.001$), but more at 20 Hz (to 8.7±5.9%, $p<0.001$). These data suggest that D3 receptor pathways play an important role in the FDP of the LLR response. We next tested how DA signaling interacts with FDP of the LLR response in WT and D3KO. We did not observe any significant changes between WT and D3KO after application of low DA (<5 μM) or the D2-receptor agonists bromocriptine and quinpirole, however, application of the D1-receptor agonist, SKF 38393, resulted in a reduced inhibition of the LLR in WT, but not D3KO. In contrast, pregabalin, which binds to the alpha-2-delta subunit of voltage gated Ca2+ channels, induced a stronger decline of LLR responses in D3KO than in WT, but resulted in similar LLR amplitudes at the end of the stimulus runs in both strains. Our data suggests that the frequency-dependent plasticity of C-fiber associated longer-latency reflexes in the spinal cord is mediated at least in part through D3 receptor pathways, and that this modulation is dependent on the activity of the Ca2+ channel alpha-2-delta subunit.

Alteration in GSK-3β^{P}and Nocioceptive Expression Following Excitotoxic Spinal Cord Injury

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Spinal cord injury (SCI) is often associated with altered sensation, called dysesthesias which result in both stimulus evoked and spontaneous pain at and below the level of the lesion. Abnormal neuronal sprouting of nocioceptors in the spinal cord and dorsal root ganglia (DRG) have been implicated in these sensory anomalies. Glycogen synthase kinase-3β (GSK-3β) is a protein highly expressed in neurons, involved in regulating neurite growth via phosphorylation (GSK-3β^{P}). Using an excitotoxic SCI model we investigated the expression pattern of GSK-3β^{P} in DRG. We examined differences in GSK-3β^{P} immunoflourescent staining intensity after spinal cord injury, and the extent to which GSK-3β^{P} co-localized with pain related proteins in the DRG. Spinal cord injury via quisqualic acid (QUIS) injection into the deep dorsal horn of the spinal cord or saline injection (control) was performed on male, Long-Evans rats. Animals were sacrificed on day 14 after injury; DRG caudal to the level of injury were isolated and fixed. DRG were cryosectioned and processed for immunoflourescent staining. Sections were double immuno-labeled for changes in GSK-3β^{P} and nocioceptive immunoreactivity using antibodies to isolectin B4 (IB4) and calcitonin gene-related peptide (CGRP) (markers for nocioceptive neurons) for co-localization studies.

Double immunofluorescence staining showed that GSK-3β^{P} was expressed entirely in DRG neurons, particularly in IB4-postive neurons. SC injured animal DRGs demonstrated a 78% increase in GSK-3β^{P} fluorescence compared to saline injected control animals. A similar and more robust increase in IB4 staining (171%) was observed in DRG tissue from SCI animals compared to saline injected controls. Increases in GSK-3β^{P} may contribute to abnormal nocioceptive neuron responses leading to pain and sensory dysfunction. Elucidating the relationship of GSK-3β^{P}upregulation and nocioceptive expression may offer a direct therapeutic target for the treatment of sensory dysesthesias following spinal cord injury. This research was funded in part by the Wooten Foundation and by ECU's Department of Research and Graduate Studies.

Role of Central Atypical Cannabinoid Receptor GPR18 in Modulating Cardiovascular Function

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Background and objectives: Recent studies have shown that GPR18 is the abnormal cannabidiol (Abn CBD) receptor that mediates vasodilation produced by abnormal cannabidiol. N-arachidonoyl glycine (NAGly), an endogenous lipid belonging to the eicosanoid super family is the endogenous ligand of GPR18 and is abundantly present in the CNS. NAGly was shown to cause microglial migration through GPR18 and has also been implicated in pain and inflammation. Given involvement of GPR18 in the endocannabinoid and peripheral vascular systems, we sought to investigate whether it is present in the cardiovascular regulatory nuclei of the brainstem and if its activation in the brainstem produces hemodynamic effects. **Key results:**Our immunofluorescence studies were the first to indicate that GPR18 is expressed in the rostral ventro-lateral medulla (RVLM), which is one of the cardiovascular regulating nuclei in the brainstem. Hemodynamic studies were conducted in conscious male Sprague Dawley rats and GPR18 agonist (Abn CBD) and antagonist (O-1918) were microinjected in to the RVLM. We show for the first time that activation of central GPR18 with Abn CBD produces a reduction in blood pressure while GPR18 antagonist, O-1918 caused an elevation in blood pressure when compared to the vehicle control. **Conclusions and implications:** The present studies indicate the expression of GPR18 in the RVLM and that it plays a role in modulating central cardiovascular functions. The present integrative studies support a favorable cardiovascular role for the putative cannabinoid receptor GPR18. Ongoing studies will elucidate the possible mechanism(s) of the hypotensive response mediated by central GPR18 activation. The findings might yield insight into the development of novel therapeutics for the management of hypertension.

The Effect of Race, Gender, and Ear on Transient Evoked Otoacoustic Emissions

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Transient evoked otoacoustic emissions (TEOAEs) are sounds emitted following acoustic stimulation to short/brief duration stimulus. TEOAEs provide a simple, efficient, and non-invasive objective indicator of healthy inner ear function. A number of factors are known to affect the amplitude of the TEOAEs including ear, gender, and age. One little explored factor affecting TEOAEs is race. African TEOAE amplitude levels have been reported to be significantly larger than Caucasians (Driscoll et al., 2010), however, the data were not subjected to inferential analysis. Herein, the effect of race on TEOAE levels was reexamined. Specifically, the purpose of the study was to examine race (i.e., African American *vs*. Caucasian), gender, and ear on TEOAE amplitude. Age, gender, and audiometrically matched normal-hearing African American (n = 12) and Caucasian (n = 14) young adults participated. There was no significant difference in TEOAE amplitude as a function of race or gender (p > .05). TEOAE levels were significantly larger in the right ear (p = .017). These findings do not support the notion that different clinical normative data be established for African American and Caucasian young adults.

Altered Expression Of Glycogen Synthase Kinase3β In The Dorsal Column Of The Spinal Cord Is Associated With Dysesthesias and Neuropathic Pain Following Excitotoxic Spinal Cord Injury

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Sensory dysesthesias and pain are common sequelae following spinal cord injury (SCI) that are associated with structural remodeling within the dorsal horn of the spinal cord. Glycogen synthase kinase-3β (GSK-3β) plays a critical role in regulating neuronal growth and survival when phosphorylated and inactivated at Ser 9 (GSK-3bP). Recent evidence suggests that GSK-3bactivity is altered after SCI. The purpose of this study was to investigate the localization of GSK-3bP in the spinal cord, examine differences in GSK-3bP after SCI, and to determine the extent to which GSK-3bPco-localized with nociceptive proteins in spinal cord tissue. Male, Long-Evans rats were injected with quisqualate (QUIS) (n=8) or an equal amount of saline (n=4) into the right deep dorsal horn of the spinal column. After 14 days, the animals were sacrificed and the cords were harvested at the level of the lesion (T12-L1). Spinal cord tissue was fixed in paraformaldehyde and cryosectioned. Tissue was double immuno-labeled with GSK-3bP and Isolectin B4 (IB4), calcitonin gene-related peptide (CGRP) (markers for nociceptive neurons), and neurofilament-200 (NF200) (marker for mechanoreceptive neurons). Images were captured and analyzed for co-localization and changes in GSK-3bPfluorescent intensity using Image Pro and Image J software.

Immunoflourescent staining showed that GSK-3bP colocalized with nociceptive markers CGRP and IB4 in the superficial dorsal horn. Spinal cord injured animals showed an increase in GSK-3bP (39.6%) and IB4 (81.3%) staining in the right superficial dorsal horn compared to saline injected controls. Interestingly, a similar trend of increased GSK-3bP (86.9%) and IB4 (73.3%) immunoreactivity in the superficial dorsal horn was observed contralateral to the side of injury. Neuropathic pain symptoms are typically resistant to standard therapies and pose a significant problem. The localization of GSK-3β to pain mediating regions of the spinal cord makes it a potential therapeutic target to resolve chronic pain following SCI.

Distribution of the Na, K-ATPase Alpha Subunits in the Rat Vestibule

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The Na, K-ATPase (NKA) is essential for excitable cells that maintain hyperpolarized membrane potential as well as sodium and potassium concentration gradients. The distribution of the NKA in the inner ear has been studied most extensively in structures that maintain the spiral ligament; however, previous work also identified the NKA alpha 3 subunit in afferent and efferent terminals and also the NKA alpha 1 subunit in GLAST-expressing supporting cells of the cochlea. These findings, in addition to the recent characterization of molecular “microdomains” in the vestibular afferent calyx ending, motivated comparable examination of NKA alpha subunits in the vestibular periphery. Therefore, we performed a variety of double labeling experiments with antibodies against three of the alpha isoforms of the NKA (NKA alpha 1-3) and markers identifying particular subsets of neurons or cells in whole mount preparations of vestibular tissue (cristae and utricle) from rat. We observed immunoreactivity against the NKA alpha 3 and 1 subunits, but not the NKA alpha 2 subunits, in calyces surrounding type I hair cells, with localization to distinct domains of the afferent calyx. No NKA alpha immunoreactivity was observed in bouton terminals contacting type II hair cells. These findings suggest unique mechanisms to regulate neuronal excitability and regulate glutamate transport between the cochlea and vestibule.

Multi-walled Carbon Nanotubes Inhibit Regenerative Axon Growth of Dorsal Root Ganglia Neurons in Mice

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Recent evidence suggests that nano-scaled particulate matter can penetrate the blood-brain barrier, affecting brain signaling pathways that are linked to Alzheimer's and Parkinson's disease. It has also been observed that due to their size, nanoparticles may exhibit greater toxicity to human tissue and cell cultures, resulting in increased oxidative stress, inflammatory cytokine production and cell death. However, the mechanism of nanomaterial toxicity on the nervous system has been poorly investigated. Addressing the question of what changes nanoparticles trigger in nerve cells could lead to discovering detectors that arise before the manifestation of physiological defects.

In our research we examined if direct and indirect exposure of primary neuronal cell cultures to nanoparticles may compromise regenerative axon growth. The regenerative response was induced by a sciatic nerve crush five days prior to the collection of dorsal root ganglia (DRG). DRG were cultured at low density and incubated overnight with different concentrations of multi-walled carbon nanotubes (MWCNT). Analysis of results indicated a significant decrease in axonal length and branching after direct exposure of DRG cultures to MWCNT in comparison to cultures incubated with the vehicle (10% surfactant). Neurite growth showed a significant decrease at 1 μg/ml while axonal branching significantly decreased at a 5 μg/ml MWCNT concentration.

We also examined if indirect exposure to MWCNT would result in a toxicological impact on the nervous system. One day following the sciatic nerve crush, animals received different doses of MWCNT through oropharyngeal instillation. The mice were sacrificed and collected for primary neuronal culture 5 days after sciatic nerve crush. Results analyzed from cell culture data indicated that indirect exposure to MWCNT also showed significant decrease in axonal length and branching at 2 μg/ml and 4 μg/ml, respectively.

A separate group of mice were used to examine the indirect effects of MWCNT in sciatic nerve regeneration *in vivo.* These mice were exposed to sciatic nerve injury and then instilled with MWCNT at a concentration of 4 μg/g bodyweight. The functional recovery after nerve injury was examined at several distinct time points (2, 4, 7, 14, & 21 days) using von Frey test and a walking track assessment. Results indicated that mice instilled with MWCNT showed a decrease in trend for restoration of sensory and motor function in comparison to mice instilled with the vehicle (10% surfactant), but the results were not significant. The cell culture results from direct and indirect exposure of MWCNT indicates that nanoparticles may have a detrimental effect on regenerative axon growth and may potentially trigger axonal pathology.

Transgenerational Effect of Paternal High-fat Diet and Exercise on Metabolic Profiles in Mouse Offspring

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Obesity and diabetes are the biggest public health concerns of the twenty-first century. Studies have identified a powerful correlation between obesity and the development of diabetes mallitus type 2. This association has elicited exploration of contributing factors, such as genetic and non-genetic transgenerational effects. Previous research shows support that the maternal diet plays an important role in offspring obesity and metabolic dysfunction; however, paternal influence remains unclear. Our model examines the epigenetic mechanisms of a paternal high-fat diet on the predisposition of offspring to obesity and glucose intolerance. C57 Black male mice were separated into 3 diet-based groups at 4 weeks of age (60% fat, 10% fat, and 10% fat with exercise). After 12 weeks of regulated diet, male mice were mated with control females. F1 offspring were then assigned to either a 60% or 10% fat diet for 12 weeks. Parameters measured postnatally at 4, 10, and 16 weeks in F0 and F1 generations include: plasma insulin and glucose metabolism (glucose tolerance test, GTT), body composition (eMRI assessment of lean/fat mass), energy expenditure (5 days housed in metabolic cage to assess motor activity, respiration, and dietary consumption), and developmental monitoring (assessed each day prior to weaning). Analysis of developmental patterns revealed impairment of upper and lower incisor development in fat fathers (FF) offspring, as well as postponed eye-opening. GTT analysis included all groups at each time point. As expected, after 12-weeks of dietary regulation the AUC of FF group was significantly elevated compared to both control fathers (CF) and exercise fathers (EF). Further observations revealed intriguing variations amongst the offspring groups. At 4 weeks of age, male offspring from EF showed notable increase in GTT, analyzed by area under the curve (AUC). At 16-weeks of age there was also a notable increase in lean mass of EF offspring versus CF offspring. The male offspring from FF on control 10% fat diet showed a significantly higher GTT in comparison to offspring from CF at 16 weeks of age. However, male offspring from FF on 60%-fat diet showed markedly diminished AUC in comparison to their fathers. Our findings indicate that prolonged paternal high-fat diet or physical activity can induce epigenetic changes in metabolic profiles of successive generation.

Injury-Induced MicroRNAs 744 and 431 Promote Regenerative Axon Growth in Murine Dorsal Root Ganglion Neurons

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MicroRNAs (miRNAs) are small, non-coding RNAs that function as important post-transcriptional regulators in a variety of developmental and physiological processes. Dysregulation of miRNAs have been implicated in many neurological disorders, such as Alzheimer's disease, Huntington's disease, and Parkinson's disease. The investigation of miRNAs expression and function has important implications for understanding disease mechanism and developing potential therapeutic intervention. Our previous work showed that peripheral nerve axons *in vivo* and *in vitro* contain functional miRNA machinery proteins that would respond to peripheral nerve injury. In addition, we demonstrated that the deletion of Dicer, a key enzyme responsible for generation of miRNAs, would delay regenerative axon growth *in vivo* and *in vitro*. These studies have indicated that miRNAs are likely to be important mediators of neuronal plasticity during peripheral nerve regeneration. After injury, both morphology and physiology of peripheral neurons undergo a number of dramatic changes aimed at promotion of axon regeneration. In current studies, we hypothesized that some of the miRNAs may be the key regulatory switches of these changes. Conditioning sciatic nerve lesion was performed on 8 week old CD1 male mice 5 days before the collection of dorsal root ganglia (DRG). MiRNA array data from injured versus naïve DRG neurons revealed a group of injury-regulated miRNAs. In particular, miR-431 and miR-744 were found to be highly up-regulated in DRG neurons following injury in microarray analyses, and in real-time qPCR experiments. In dissociated DRG neurons, the gain and loss of function analyses for miR-431 and miR-744 were performed by applying Pre-miR miRNA precursors and Anti-miR miRNA inhibitors. These experiments demonstrated that overexpression of miR-431 and miR-744 promoted neurite outgrowth. PCR-array experiments also revealed that several genes playing important roles in neurogenesis altered their expression level in response to overexpression of miR-431 and miR-744. Potential gene targets for miR-431 and miR-744 were predicted by web-based bioinformatics tools. The predictions were validated by the inverse correlation between the expression of the predicted target genes and their miRNAs using RT-qPCR . The direct interactions between miRNA and mRNA of the target genes were confirmed by cross-linked immunoprecipitation. These observations supported our hypothesis of miRNAs' critical role in peripheral nerve regeneration. The current data provided further evidence that miR-431 and miR-744 could be promising candidates for the future miRNA based therapies that enhance the endogenous neuroregenerative and/or neuroprotective capacity.