SYNAPSE 2007

Symposium for Young Neuroscientists and Professors of the Southeast

Abstracts

Poster #01

***Anderson MA, Ringler SL, Turner CP**

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LOWERING INTRACELLULAR CALCIUM LEVELS DISRUPTS EARLY DEVELOPMENT OF EMBRYONIC RAT NEURONS

Early neuronal development, characterized by growth cone expansion, axonal branching and extension, and signal transduction, is regulated by intracellular calcium levels. There are multiple sources for calcium ions including the influx of extracellular calcium through ion channels and ionotropic receptors such as the NMDA receptor as well as internal calcium stores in the endoplasmic reticulum. Reducing intracellular calcium levels by disrupting these sources, therefore, may interfere with normal neuronal development. To explore the effects of decreased intracellular calcium, the growth of embryonic day 18, rat cortical neurons was monitored in the presence and absence of MK801, an NMDA receptor blocker, nimodipine, an L-type Ca2+ channel blocker, thapsigargin, a Ca2+-ATPase pump inhibitor, and BAPTA-AM, a chelator of cytoplasmic Ca2+. Cell cultures were treated with these agents for 24 hours at 1 DIV and then fixed and processed on the following day, labeling for actin with rhodamine phalloidin, for axonal and dendritic proteins with MAP2 and tau, and for nuclei with DAPI. We found that all these agents inhibited morphological maturation events such as growth cone expansion as well as neurite lengthening and branching, suggesting that normal neuronal development is dependent on the maintenance of intracellular calcium homeostasis. Future studies will explore the ability for neurons to recover from exposure to these agents and regain elements of normal development.

Poster #02

***Atukorale VN, Greene MW, Silver WL**

Department of Biology, Wake Forest University

ARTIFICIAL SWEETENERS, NACL, AND KCL STIMULATE TRIGEMINAL NERVE CHEMORECEPTORS

The trigeminal nerve provides sensory information from the eyes, nose, and mouth. It is a multisensory nerve, responsive to a variety of irritants in the environment. Several receptor proteins associated with the trigeminal nerve mediate these responses. Artificial sweeteners, which are of obvious economic importance, are reported to produce irritation at high concentrations. The present study examines peripheral trigeminal nerve responses to increasing concentrations of three artificial sweeteners (Sodium Saccharin, Sodium Cyclamate, and Acesulfame K), NaCl, and KCl. These stimuli were injected into Ringer's solution that was pumped continuously through the nasal cavity of anesthetized adult Sprague-Dawley rats. Multiunit neural activity from the ethmoid branch of the trigeminal nerve was summated using an averaging circuit. The data were analyzed by taking the maximum height of the integrated response after stimulus delivery, and these results are reported as percentages of the standard response to 10µM capsaicin. The results show that the artificial sweeteners, NaCl, and KCl elicited graded responses (with respect to increasing concentrations) from the ethmoid branch of the trigeminal nerve. Although Na+ and K+ may have played a role in the response to the three artificial sweeteners tested, the thresholds for NaCl and KCl were higher than those of the sweeteners, suggesting that the sweeteners must be stimulatory by themselves. Previous studies suggest that artificial sweeteners may activate TRPV1 (irritant, capsaicin) or T2R (bitter) receptors. Future experiments will examine trigeminal nerve responses to the sweeteners in the presence of TRPV1 and T2R receptor blockers.

Poster #03

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DEVELOPMENTAL SWITCH IN CALCIUM BINDING PROTEIN EXPRESSION: CINGULATE CORTEX

Our lab has previously shown that age-dependent, MK801-induced, activated caspase-3 expression is generally not observed in neurons expressing calcium binding proteins (CaBPs). This suggests that cell death and calcium buffering are inversely related in animals treated with agents that reduce intracellular calcium. Because in vivo injury peaks at postnatal day 7 (P7) and rapidly diminishes thereafter, we hypothesized that postnatal changes in CaBP expression may coincide with loss of MK801-sensitivity. In the cingulate cortex (a region sensitive to NMDAR blockade at P7), expression of calbindin-D28K (CB) and calretinin (CR) was relatively low from P0-P7, steadily increased from P7-P14 and declined (rapidly in the case of CR) at ages thereafter. Parvalbumin (PV) was low or absent prior to P7 but expression dramatically

increased from P10 onwards. Thus, postnatal changes in CaBP expression (in particular PV) coincided with loss of MK801-sensitivity. Our observations are important for understanding normal central nervous system development and its ability to deal with trauma. Also, given that schizophrenia is associated with developmental brain injury, loss of glutamatergic activity, and changes in expression of CaBPs, our observations may be clinically relevant.

Poster #04

***Biancaniello S M, Zvara DA, Bryant AJ, Deal DD, Demarco MP, Campos KM, Mansfield CM, Tytell M**

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SPINAL CORD INJURY AND THE ROLE OF THE 25 KD HEAT SHOCK PROTEIN

The purpose of our study is to test ways to protect the spinal cord from injury, specifically the "stress protein response", and the neuroprotective effects of one heat shock protein (Hsp25). We hypothesized that neurologic damage would be reduced after ischemic preconditioning (IPC), and both acute and chronic anesthetic preconditioning (APC). APC was done with sevoflurane, a volatile anesthetic shown to make brain and heart tissue more resistant to ischemic injury. Rats were randomized into 5 treatment groups. The IPC group received 3 minutes of IPC, then 12 minutes of induced ischemia. The chronic APC group (cSEVO) had one hour of APC for 2 days before ischemia, and the acute APC group (aSEVO) had one hour of APC then a one-hour washout period before ischemia. The control group did not receive any preconditioning, and the sham group underwent the same surgery without ischemia. To perform histological analysis, cervical segments (uninjured control) and lumbar sections (effected by the ischemia) of the spinal cord were embedded onto slides. Later the sections were deparaffinanized and stained with anti-Hsp antibody to detect immunofluorescence histochemistry (IHC). In normal spinal cord, the greatest amount of Hsp25 was found in large neurons like motor neurons in ventral gray matter, while injured tissue showed more neuronal damage and less Hsp25. IPC treatment increased Hsp25 in injured segments, indicating neuroprotective benefit in motor neurons/reduction of paralysis. Also, neither APC reduced damage significantly, and had less Hsp25 present than the IPC group. Interestingly, the APC segments seemed to show an increase in glial cells in the white matter

Poster #05

***Black LC, *Walton AL, Gazda RL, Kelly SJ**

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DEVELOPMENTAL ALCOHOL EXPOSURE AND EFFECTS ON ATTENTIONAL SET SHIFTING IN LONG EVANS RATS: A PILOT STUDY

Attentional set shifting has been proven to provide insight into specific types of damage to the prefrontal cortex, an area of the brain involved in executive control such as decision making and ability to appreciate the consequences of one's actions. Set shifting in humans is often evaluated using the Wisconsin Card Sorting task. A cross species equivalent has been developed to test rats by Birrell and Brown (J Neurosci 20:4320-4324, 2000). The purpose of this study was to determine whether this behavioral task is useful in assessing damage to the prefrontal cortex caused by developmental alcohol exposure in an animal model of Fetal Alcohol Spectrum Disorder. A non-treated control, an intubated-control, and an ethanol-treated group of Long Evans rats were used. The rats performed a set shifting task in which they were trained to dig in bowls for a food reward. Then, they had to learn to discriminate between two different bowls—only one of which had the food reward in it—based on different odors, mediums, or textures. A series of discriminations were tested, including intra-dimensional shifts, extra-dimensional shifts, and reversals. All rats in a pilot study were able to fully complete the set shifting task and were fast learners overall. Thus, we expect that this attentional set shifting task will be a good indicator of damage to the prefrontal cortex that may be due to developmental alcohol exposure. Supported by NIAAA grant 11566.

Poster #06

***Bonner HC, Lawrence RC, Kelly SJ**

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NEURONAL ACTIVATION AS MEASURED BY C-FOS IMMUNOREACTIVITY BY PLAY BEHAVIOR IS ALTERED IN RATS EXPOSED TO ETHANOL DURING DEVELOPMENT

Perinatal ethanol exposure in rats significantly affects social interaction, a finding which parallels effects seen in Fetal Alcohol Syndrome (FAS) in humans. Play behavior, a social interaction consisting of dorsal contacts and pinning in rats, has been shown to activate *c-fos*, an indicator of neuronal activity, in a number of brain regions. The aim of this study was to investigate neuronal activation in perinatal ethanol-exposed rats following play behavior by analyzing *c-fos* immunoreactivity (*c-fos* IR). In this study, rats were allowed to play together for one hour in a closed field. Immediately following the play bout, rats were anesthetized and perfused. Their brains were sliced, stained, and analyzed for presence of *c-fos* IR. This study found that there were no significant differences among groups in the amount of *c-fos* IR in the striatum, nucleus accumbens, or prelimbic cortex. However, *c-fos* IR was significantly decreased in the primary somatosensory cortex (S1sh) of ethanol- treated rats, as compared to non-treated and intubated controls. The significantly smaller amount of *c-fos* IR in the S1sh of ethanol rats indicates less neuronal activation of this region during play behavior. A companion study has shown that ethanol-treated rats have significantly more pins during a play bout and that this behavior in the ethanol-treated rats is more sensitive to somatosensory degradation compared to control rats. Taken together, perinatal ethanol exposure causes a deficiency in somatosensory signal processing resulting in social interaction deficits as evidenced by abnormal play behavior. Supported by NIAAA 11566 to S.J.K.

Poster #07

***Boothe E, Roender C, Ruscio MG**

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NEUROGENESIS ASSOCIATED WITH PARENTAL RESPONSIVENESS IN PRAIRIE VOLES

Rates of adult neurogenesis in mammals are known to be affected by social environment, stress and learning. However, potential relationships between parental care and neurogenesis have not been fully explored. In the present experiment we measured rates of neurogenesis in male and female adult virgin prairie voles (Microtus ochrogaster) in response to foster pup exposure and subsequent degree of parental responsiveness. Prairie voles are highly social rodents which typically display biparental and foster parental care. Following a 20 minute exposure to pups (or control conditions) we quantified BrdU immunoreactivity in the dentate gyrus of the hippocampus. The dentate gyrus is an established proliferative zone where neurogenesis occurs in adult mammals. BrdU is a marker of cellular division. Using double label immunofluorescence we identified the phenotype of BrdU labeled cells as either neuronal or glial. Males and females exposed to pups showed a greater number of BrdU labeled cells than control groups. The degree of parental responsiveness inversely affected the number of BrdU labeled cells. Proportions of neuronal versus glial cells in the dentate gyrus were not different among groups.

Poster #08

***Bowden CN, Rogers JO, Cline MA**

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CENTRAL OXYNTOMODULIN DECREASES FEED INTAKE IN 4-DAY POST HATCH CHICKS

Oxyntomodulin (OXM), a 37 amino acid neuropeptide reduces feed intake in rats when injected centrally. The biological role of OXM in avians is unreported. Thus, 4 days-post hatch chicks were randomly assigned to receive 0, 3.0, 6.0, or 12.0 µg of rattus OXM. Rat and chicken OXM share 93% sequence identity. Following injection, feed intake was monitored every 30 min for 180 min. OXM-treated chicks responded with a linear dose-dependent decrease in feed intake. To determine if the hypothalamus mediates this effect, chicks were injected with 6.0 µg OXM and c-Fos immuno-reactivity was quantified in the lateral hypothalamus, paraventricular nucleus, and ventromedial hypothalamus. Treatment did not effect neuronal activation. To determine if OXM affects gastrointestinal dynamics, chicks randomly received 0, 3.0, 6.0, and 12.0 µg OXM and were gavaged with a ferric oxide containing feed. Time the distinctive ferric oxide color was visually detected in feces was determined. Central OXM did not affect gastrointestinal transit rate. We conclude that central OXM reduces feed intake in chicks; however, the effects do not appear to be mediated via the hypothalamus or gut.

Poster #09

***Boyd JL, Daniels J, Warrick J**

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LOCALIZATION OF CREB-BINDING PROTEIN (CBP) IN PATHOGENIC AND RESCUED MODELS OF MJD POLYGLUTAMINE DEGENERATION

Machado-Joseph disease (MJD), which is also called spinocerebellar ataxia type 3 (SCA-3), is a progressive neurodegenerative disease. Disease development is associated with formation of insolulable nuclear inclusion bodies. It is hypothesized that MJD pathology may be associated with aberrant cellular localization of transcriptional regulators. One such regulator is the transcription cofactor CREB-Binding Protein (CBP). CBP has been found in aggregates of mutant polyglutamine proteins. The sequestration of this transcription factor into the insoluble aggregates could result in transcriptional dysregulation. Our studies sought to assess the effect of co-expression of human MJD with heat shock chaperones, which are known to rescue MJD pathology, on aggregate formation and localization of CBP. Analysis of rescued and non-rescued models showed differential CBP localization and aggregate formation.

Poster #10

***Buff H, Fowler E, Korey C**

Department of Biology, College of Charleston

A DOMINANT LOSS-OF-FUNCTION SCREEN FOR MODIFIERS OF PPT1-INDUCED DEGENERATION

Infantile Neuronal Ceroid Lipofuscinosis is caused by mutations in the cln1 gene which encodes palmitoyl-protein thioesterase 1 (Ppt1), suggesting that there is an important role for the regulation of palmitoylation in normal neuronal function. Loss of Ppt1 function in patients produces granular osmiophilic deposits (GRODS) in all cells and massive neurodegeneration. Consistent with an important role for PPT1 in neurons, the protein is found with synaptic vesicles and synaptosomes in neuronal cell culture. Recently, we reported the results of a gain-of-function modifier screen in which we used ~2000 EP and EY transgenic overexpression lines to suppress or enhance the degeneration produced by Drosophila Ppt1 overexpression. The 20 modifier genes we identified further link Ppt1 function to synaptic development and function. We are now performing a second, dominant loss-of-function screen to gain further insight into Ppt1 function. We will present the screen's methodology and our preliminary results.

***Carroll KJ, O'Donnell B, Payne C, Lom B**
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THE PESTICIDE MALATHION REDUCES NEUROGENESIS IN ZEBRAFISH

Malathion is an organophosphate insecticide designed to control mosquitoes, flies, boll weevils, and lice. It is an acetycholinesterase (AChE) inhibitor that disrupts nervous system function by enhancing acetylcholine (ACh) activity in the synaptic cleft. Malathion contaminates water through aerial application, runoff, and erosion and therefore can exert harmful effects on non-target aquatic organisms such as fish and amphibians. We used islet-1 GFP zebrafish to study the effects of malathion on early vertebrate neurogenesis. Islet-1 GFP zebrafish express green fluorescent protein (GFP) in a subset of neurons. In the spinal cord, GFP is first expressed by secondary motoneurons and interneurons. We treated islet-1 GFP zebrafish embryos with 2.5 mg/L of malathion, vehicle (acetone), or embryo media for 48 hours. We then examined the number and position of GFP expressing secondary motoneurons and spinal cord interneurons in a segment of the tail just posterior to the gut using confocal microscopy. We observed significantly fewer total neurons in the embryos treated with malathion compared to both vehicle and dilution controls, suggesting that this pesticide compromises early neurogenesis in the spinal cord. Closer examination reveals that malathion preferentially reduces secondary motoneurons without affecting interneurons. Malathion's reduction in neurons was accompanied by a significant reduction in somite area as well as a reduction in neurons per somite. Vertebrate neurogenesis occurs in an anterior to posterior fashion, but malathion did not differentially affect anterior versus posterior neurogenesis. Taken together, our results indicate that changes in cholinergic activity can compromise early neurogenesis in the spinal cord.

***Cribb J, *Ou N, Norflus FN**
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ANALYSIS OF SENSORY RECEPTOR BEHAVIOR IN A C. ELEGANS MODEL OF HUNTINGTON'S DISEASE

Huntington's Disease (HD) is a neurodegenerative disorder affecting approximately 30,000 people in the United States. Symptoms of HD include mood swings, involuntary movement, and lack of concentration. The disease is characterized by a mutation in the HD gene causing expanded CAG repeats which result in an abnormally long polyglutamine tract in the huntingtin protein. This causes the protein to fold incorrectly, forming clumps that prevent the cell from performing its normal function. Our lab studied HD in *Caenorhabditis elegans.* We performed two behavioral assays, a nose touch assay and an osmolarity assay. We studied *C. elegans* wild type (N2), *C. elegans* with non-lethal HD (HDQ150), and *C. elegans* with lethal HD (HDQ150 OSM-10::GFP). For the nose touch assay, we placed an eyebrow hair in front of an individual worm so that the worm would run into the hair at a 90° angle. The worms were described as responsive if they immediately recoiled from the hair. We found that 54% of N2 strain responded to nose touch, 45% of HDQ150 strain responded, and 1% of HDQ150 OSM-10::GFP strain responded. The osmolarity assay measured the ability of the worms to cross an osmotic barrier. Using 8M glycerol, we created an osmotic ring and placed the worms in the center. We allotted 60 minutes for movement, and then we measured the amount that crossed the barrier versus those that remained in the ring. Data retrieved showed that the HDQ150 OSM-10::GFP strain crossed the barrier two folds more then the N2 strain.

***Goldston Amanda M; Mitchell DJ; Rife TK**
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CHARACTERIZATION OF A POLYMORPHISM IN THE NITRIC OXIDE SYNTHASE 1 1F PROMOTER

Nitric Oxide Synthase (NOS), which produces nitric oxide, exists in three major forms: neuronal, endothelial, and inducible. Neuronal (NOS1), which is the focus of this research, has been shown to affect learning, immune system response, and even sleep patterns. Upregulation of NOS1 is believed to lead to neural degeneration following stroke and spinal cord injury. The enzyme has also been linked to the severity of symptoms of both Cystic Fibrosis and Parkinson's disease. A study on one of the eleven promoters of NOS1, 1F, showed that a TGnTATGn polymorphism present in this promoter (ranging from 40 to 62 bp in length) is shorter in some Parkinson's patients than in age-matched controls. To test the effects of varying polymorphism length, two reporter gene constructs possessing TGnTATGn repeats varying in length by 20 base pairs were prepared. When these two constructs were expressed in HeLa cells, a two-fold increase in the expression of the longer polymorphism compared to the shorter was observed. There is a TATA box downstream from the polymorphism and a repressor region is located upstream of the polymorphism in the 1F promoter. It was hypothesized that the movement of the repressor site further from the TATA box may be responsible for the increased reporter gene expression. Alternatively, there may be a transcription factor binding to the polymorphism itself that affects the rate of transcription. Reporter gene constructs were created to test these two hypotheses. These constructs will be placed into both HeLa and Neuroblastoma (SK-N-MC) cell lines along with a Beta-galactosidase reporter gene in order to quantify the amount of activity shown.

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RELATIVE DEPRIVATION AND ENVIRONMENTAL CONTEXT: EFFECT OF SOCIAL STRESS ON WOUND HEALING IN RATS

Relative deprivation is the perception that one is less well off than others to whom one compares oneself. In humans, relative deprivation may have a possible role in long-term illness and mortality levels in certain non-migrant populations. There is evidence that rats placed in enriched environments demonstrate changes in several physiological measures as compared to controls. We sought to create an animal model of relative deprivatino by measuring the effect of environmental context on a single physiological measure- wound healing. Two female Sprague-Dawley rats (Enriched Environment-EE) were placed in a large enclosure (152.4 cm x 76.84 cm x 41.28 cm) containing tubes and blocks that were changed periodically. The rats had free access to food and water. In addition, flavored cereal was placed on the walls of the enclosure in different positions, once a day for a period of six weeks. Within the larger enclosure was a smaller cage (47.63 cm x 25.4 cm x 20.32 cm) which contained 2 other female rats (Relative Deprivation- RD). The RD rats had free access to normal rat chow and water. At the end of the treatment period, each rat was wounded and photos of the wound were obtained via digital camera over three days. Rate of healing as compared to the original wound size was used as a measure of comparison. The results reveal no significant difference between groups. However, when results were compared to a previous pilot study using male rats a possible gender-based difference was revealed. An investigation of gender differences in an immune response to non-physical social stress will be pursued further by considering the effects of environmental context on cytokine levels in male and female rats.

***Harrold JB, Parise MA, CL Cleland**
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THREE DIMENSIONAL MOVEMENT ANALYSIS OF THE CHARACTERISTICS AND HABITUATION OF TAIL WITHDRAWAL RESPONSES TO PINPOINT HEAT STIMULI IN THE RAT

Previous results from our laboratory revealed that heat stimuli applied to the rat tail evoked withdrawal movements whose direction depended on the location of the stimulus. These results were obtained by recording isometric force or the initial component of the movement. Both of these approaches, however, fail to capture the complex 3-dimensional nature of the withdrawal response. The goal of these experiments was to quantify the withdrawal response of the rat tail using high-speed, 3-dimensional video. Rats were restrained vertically and the tail was marked 1/3 of the distance from the body for stimulation and movement tracking. Stimuli (laser; 2 mm and 1mm diameter) were delivered to the right side of the tail at 5 minute intervals (total 25 trails). Movement was recorded with two high-speed video cameras (250 fps). The direction, speed and magnitude of movement were quantified. Results showed the initial movement was in the dorsolateral direction, consistent with previous studies. The initial response was observed to be followed by a strong ventral or left-ventral flexion of tail. These two responses also differed in the way they were produced. The early response arose from local bending just above the point of stimulation, while the lower tail remaining largely passive. The second, ventral response, however, arose from pronounced bending of the entire tail. When using 2 mm diameter stimuli the direction, velocity and magnitude of response were stable. However, in experiments with 1 mm diameter stimuli there was significant habituation in movement velocity and magnitude, and direction shifted from dorsolateral to lateral. These results suggest that the tail withdrawal reflex in the rat utilizes a complex, multiphasic strategy to avoid injury. Funded by NSF.

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SOCIAL AND ENVIRONMENTAL ENRICHMENT INCREASES COCAINE SELF-ADMINISTRATION IN FEMALE RATS

Previous studies conducted in male rats have reported that social and environmental enrichment enhances sensitivity to the locomotor and rewarding effects of psychomotor stimulants. Relatively few studies have examined these types of manipulations in female rats, and thus it is not known whether females are similarly sensitive to these effects. The purpose of the present study was to determine whether social and environmental enrichment enhances sensitivity to the positive-reinforcing effects of cocaine in female rats. Rats were obtained at weaning and randomly assigned to one of two housing conditions: isolated rats were housed individually with no visual or tactile contact with other rats; enriched rats were housed in groups of four in large cages and given novel objects on a daily basis. After six weeks under these conditions, rats from both groups were surgically implanted with indwelling intravenous catheters and trained to self-administer cocaine under positive-reinforcement contingencies. Once self-administration was acquired, cocaine was made available on a progressive ratio schedule and breakpoints were obtained for various doses of cocaine in both groups of rats. Enriched rats acquired cocaine self-administration in significantly fewer days than isolated rats (6 days vs. 16 days). On the progressive ratio schedule, breakpoints were non-significantly greater in enriched rats than isolated rats when responding was maintained by both low (0.3 mg/kg/inf) and high (1.0 mg/kg/inf) doses of cocaine. These data

suggest that social and environmental enrichment enhances sensitivity to the positive-reinforcing effects of cocaine in female rats

***Jackson CM**
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CAN HONEY BEES DISCRIMINATE MULTIPLE PATTERNS?

The honey bee (Apis mellifera) is a promising model for memory and cognition as many of the processes described in the honey bee brain are also found in mammalian systems. Honey bees and other Hymenoptera are unique among insects in that their mushroom bodies (locus associated with memory and cognition) receive extensive input from the optic lobes, suggesting that vision is closely linked to learning and memory. Previous studies have revealed that bees are remarkably capable of using visual cues to solve navigation tasks. This study implemented a Y-maze paradigm to examine how bees perform when asked to successively discriminate multiple pattern sets in the absence of external context cues. Overall performance was lower when multiple pattern sets were presented successively than when a single pattern set was presented in isolation. When these pattern sets were alternated, performance exhibited a roughly direct correlation, suggesting a degree of overlap in the tuning curves of the orientation-sensitive channels that process these patterns. In contrast, when bees that had learned a grating pattern set and a set of concentric patterns were presented with these pattern sets successively, performance exhibited a roughly inverse correlation; suggesting that the pathways that process these patterns inhibit one another. Behavioral experiments such as these provide insight into brain anatomy and physiology and may be especially useful for studying experience-dependent brain plasticity in the context of memory and learning.

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IN VITRO ANALYSIS OF RETINAL GROWTH CONE DYNAMICS

Growth cones are the dynamic ends of growing axons that navigate through the brain to locate appropriate synaptic targets. Thus, growth cones play critical roles in wiring the nervous system. Cultured neurons form growth cones that move independently and dynamically on artificial substrates and are often used in studies of neuronal development and cell motility. In order to characterize Xenopus retinal neuron growth cone dynamics in vitro we analyzed time-lapse images. We prepared explants of stage 24 28 Xenopus laevis retinas and used time-lapse imaging to record retinal neuron growth cone movements for 30 minutes. We measured growth cone area and the distance traveled at two minute intervals in order to calculate the growth rate, the straightness of the growth cone trajectory, and the consistency of the growth rate. Our results demonstrate that most (>70%) retinal growth cones grow in straight trajectories, but at inconsistent intervals. Overall growth cone rates varied from 40 160 µm/hr. Moreover, we observed that lamellipodial area and time in culture did not correlate with overall growth rates. This characterization of retinal neuron growth cone dynamics will be particularly useful as a baseline for future studies examining how specific molecules influence growth cone behaviors in vitro.

***Jones LS, Warren AC, Allen L, Barrett S, Black LC, Blew M, Bright LA, Bonner HC, Desai R, Goodlett B, Guram J, Juneja N, Jones N, Khaliq Z, Kent J, Meekins C, T Meisner T, Montagu D, Moore A, Patel A, Patel D, Ryan K, Sousa B, ST**
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IMPULSE: AN ONLINE, INTERNATIONAL JOURNAL FOR NEUROSCIENCE REPORTS WRITTEN, EDITED, AND PUBLISHED BY UNDERGRADUATES.

IMPULSE is an online journal (http://impulse.schc.sc.edu) created by undergraduate students interested in neuroscience and/or publishing. It is hosted at the University of South Carolina, but also invites remote reviewers from universities around the world. The first issue was posted in 2004 (see Soc. Neur. Abs. (2003) 29:25.3 and Soc. Neur. Abs. (2004) 30:28.6). The journal shifted to a rolling submission format in '05 (see Soc. Neur. Abs. (2005) 31:20.19) and this model has been in use since (Soc. Neur. Abs. (2006) 32:26.13). All articles are written and reviewed by undergraduates. The review team comprises students from over 15 universities internationally, including the host site, the University of South Carolina. Reviewers at USC take a Scientific Publishing class when they join (described in JUNE Spring 2006 Vol. 4, Issue 2 http://www.funjournal.org/results.asp?juneid=159), promoting understanding of and commitment to the review process. The premise is that learning about publishing is an important component of a scientific education and that direct experience of this process will prove useful to faculty mentors and their students wishing to complete the progression from proposal to project to paper. Anecdotal evidence from student reviewers indicates that refereeing manuscripts promotes a practical understanding of scientific writing and publishing that will be valuable in their future careers. Faculty at other institutions are encouraged to use the course and ***IMPULSE***, or a similar model, to foster professional development in neuroscience programs. Supported by the SC Honors College.

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ASSESSMENT OF NEURODEGENERATION IN RATS FOLLOWING PILOCARPINE-INDUCED STATUS EPILEPTICUS

Status epilepticus (SE) is a neurological emergency that can cause widespread damage to the brain. In clinical studies the relationship between seizure activity and neuronal damage is difficult to investigate because of co-existing morbidity. A better understanding of brain regions damaged could lead to improved treatment and care of affected individuals. We used magnetic resonance imaging (MRI) and histological analysis to quantify neurological damage 10 days after pilocarpine-induced status epilepticus in adult rats and to determine the sensitivity of MRI in detecting this neurodegeneration. Rats were injected with either pilocarpine to produce 4 hours of SE or saline for control. Following a recovery period of 10 days 6 SE-experienced and 6 control rats were scanned in a Bruker 7T animal MR scanner, where a T2 weighted structural volume was obtained. Alternately, following pilocarpine treatment, 9 SE-experienced and 10 control rats were perfused intracardially with paraformaldehyde and their brains sectioned for histological analysis. Brain sections were stained with cresyl violet and neurons in regions of interest were counted using stereological techniques. Significant neuronal loss was observed in the hilus of the dentate gyrus (74 + 4%), areas CA1 (33 + 11%) and CA3 (36 + 10%) of the hippocampus and the piriform cortex (62 + 6%). In contrast, amygdala and the granule cell layer of the dentate gyrus did not experience significant cell loss. MRI scans exhibited a significant increase in the T2 signal, a marker of cell loss in hippocampus, medial temporal lobe and piriform cortex, but not in amygdala. These results demonstrate significant damage to select limbic structures following pilocarpine-induced SE and indicate that MRI can reliably detect this damage.

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EFFECT OF CYCLOHEXIMIDE ON EXTINCTION OF ODOR DISCRIMINATION LEARNING IN RATS

Much research has shown that protein synthesis is critical for the new learning of certain types of information (e.g., Nader, Schafe, and LeDoux, 2000). However, the results from a small number of studies examining extinction and protein synthesis have produced mixed results (Lattal & Abel, 2001; Suzuki, et al., 2004). The present study examined the effect of a protein synthesis inhibitor (cycloheximide, 1 mg/kg) on extinction of memories for an odor discrimination (Hanson, Bunsey, & Riccio, 2000). Rats learned to dig in a particular cup of sand (scented with cinnamon or cocoa) to find a buried food reward. Rats received three shaping sessions (ten trials), followed by one training session (between eight and eleven trials). One day following training, rats received extinction trials for the odor discrimination (rats received either a cycloheximide or a vehicle injection thirty minutes prior to extinction trials). One day following extinction, rats were tested for their retention of the odor discrimination with nonreinforced probe trials (two trials). Results showed no statistical differences between the cycloheximide-injected rats and controls. However, the results appear to be in the direction of the cycloheximide-injected rats being more likely to dig than the controls on the test trials (i.e., no controls dug on the test; however, several cycloheximide-injected rats did). This may indicate, in a non-statistically significant way, that the cycloheximide-injected rats may have forgotten the extinction trials from the previous day, however, further data collection will be necessary before a more conclusive statement can be made.

Lema Tomé CM, Bauer C, Nottingham C, Smith C, Blackstone K, Brown L, Hlavaty C, Nelson C, Daker R, Sola R, *Miller R, Bryan R, Turner CP
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MK801-INDUCED CASPASE-3 IN THE POSTNATAL BRAIN: INVERSE RELATIONSHIP WITH CALCIUM BINDING PROTEINS.

Age-dependent, neuronal apoptosis following N-methyl-D-aspartate receptor blockade has been linked to loss of calcium. To further explore this relationship, we examined expression of activated caspase-3, as well as the calcium binding proteins, calbindin-D 28K, calretinin and parvalbumin, following injection of vehicle or the N-methyl-D-aspartate receptor blocker, MK801, in postnatal day 7 or 21 rats. At postnatal day 7, MK801-induced activated caspase-3 expression was most frequently found in mutually exclusive cell populations to those expressing any of the three calcium binding proteins. For example, in the somatosensory cortex, most immunoreactivity for activated caspase-3 was found in layers IV/V, layered between areas of high calbindin or calretinin expression. Further, in the caudate putamen, activated caspase-3 rarely invaded zones of intense calbindin immunoreactivity. Suggesting expression patterns of these proteins were inversely related, these same bra! in regions no longer displayed MK801-induced activated caspase-3 at postnatal day 21, but instead robustly expressed calcium binding proteins. This later surge in expression was especially true for parvalbumin in regions such as the somatosensory and retrosplenial cortex, as well as the subicular complex. Calbindin-D 28K was also found to increase in the same regions though not as impressively as parvalbumin. Thus, developmental regulation of calcium binding protein expression may be a critical factor in age-dependent sensitivity to agents that disrupt

calcium homeostasis in maturing neurons, providing a possible mechanistic explanation for age-dependent MK801 toxicity.

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EFFECT OF AGE AND SEX ON ENDOTHELIN-1 INDUCED C-FOS EXPRESSION IN THE RAT BRAIN

By birth all individuals possess the anatomical structures associated with pain perception, but the level of functionality and activity across sex and development is not understood. The pain pathway involves both ascending and descending circuits that allow the body to perceive and modify pain sensations. This study analyzes the activity of various brain regions in response to acute pain resulting from an injection of endothelin-1 (ET-1) into the left plantar hindpaw of postnatal day (P) 7, 21, and 60 rats of both sexes. ET-1 release is associated with a variety of pain states including the vaso-occlusive episodes of sickle cell disease. Two hours post-injection, rats were perfused. Brains were serially sectioned and immunohistochemically stained for c-fos—a protein marker for neuronal activity. An investigator blinded to both sex and treatment counted c-fos positive neurons in the somatosensory cortex, thalamus, amygdala, hypothalamus, periaqueductal grey, and rostral ventral medulla. Preliminary analyses indicated that ET-1 increases c-fos expression in the primary somatosensory cortex, paraventricular thalamus, lateral periaqueductal grey, and central amygdala. Older animals generally had increased expression in comparison to younger animals, while some areas showed a sex dependence as well. These differences indicate changes in pain physiology across sex and development and may explain observed differences in pain behavior. Recognition and increased understanding of these differences could eventually lead to more effective and individual pain management.

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VASO-OCCLUSIVE PAIN EARLY IN LIFE INCREASES ACUTE PROCEDURAL PAIN IN THE PEDIATRIC SICKLE CELL POPULATION

Children with Sickle Cell Disease (SCD) experience significant painful vaso-occlusive episodes (VOEs). The first VOE may occur as early as six months, but age of onset, frequency, and severity vary. Studies of premature infants and male infant circumcision at birth indicate painful experiences early in life affect the developing nervous system and alter pain sensation. Little is known regarding the impact of VOEs on subsequent pain experiences. This study used a multi-method evaluation of pain in response to a routine venipuncture across three age groups (2-4, 5-9, & 13-18 years) of children with SCD. Venipuncture pain was evaluated via heart rate changes, parent and child pain reports, and observations of behavioral distress. This investigation is the first to conduct a multi-method evaluation of procedural pain across age. Change in heart rate, pain reports, and behavioral distress decreased as age increased. Data was also analyzed to compare the affect of VOE onset prior versus after 3 years. Patients whose first episode occurred in the first 3 years of life exhibited greater increases in heart rate, higher pain reports, and more behavioral distress. These data indicate venipuncture pain is greater for younger children and painful VOEs early in development sensitize a child to acute procedural pain later in life. These findings emphasize the importance of pain management during both routine painful procedures and VOEs especially in younger children.

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ESTROUS STATE AS DETERMINED BY VISUAL METHOD IN MICE

Introduction: Sex differences in behavior are well known. It is also increasingly clear that these behavioral differences are mediated by differences in gene expression, neurochemistry, and neurocircuitry (Guillamon and Segovia, 1997). Despite this fact, many behavioral studies fail to test both sexes due to the increased variability (as a result of variations in circulating steroid hormones) in females. When attempts are made to account for female hormonal influences, experimenters generally use swabbing, which often proves to be quite tedious. We found evidence that a visual method can be used that is as reliable, if not more so, than the swabbing method (Champlin et al., 1973).
Goal: A clearly defined visual method would provide a much less complicated approach to identifying estrous states in mice. Furthermore, it would allow experimenters to incorporate female subjects into studies more often, thereby accounting for sex differences and providing a realistic representation of populations.
Methods: The study used 65 naïve female Swiss Webster and Mc1R mice. Each female was looked at by three observers independently and deemed either estrus (receptive) or nonestrus (unreceptive). The mice were identified by certain criteria including swelling, color, striation, and moisture. If the three observers were all in agreement as to estrous state, a mouse was included in a separate study that day. If not, the mouse was included at a later time.
Results: The subsequent study looked at social interaction between male and female mice, while taking into account the estrous state. The results from the social interaction study were significant, hence the discrimination of estrous state was

also significant. Additionally, the average weight between estrus and nonestrus mice was significant, which provides support for the method.
Conclusion: Therefore, our findings give support to a visual method being used to successfully determine estrous states in mice.

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SPATIAL ORGANIZATION OF ESCAPE MOVEMENTS IN RESPONSE TO HEAT STIMULI IN THE CHILEAN ROSE TARANTULA

Animals exhibit escape or withdrawal responses to potentially injurious stimuli. As with all movements, animals are faced with a speed-accuracy tradeoff. In contrast to voluntary limb movements, however, escape responses may place a greater importance on speed. Since the number of degrees of freedom that must be controlled has a major impact on movement complexity, the analysis of animal movements with a large number of degrees of freedom, such as the spider, may be especially revealing as to how animals address the speed-accuracy tradeoff in escape responses. Chilean rose tarantula (Grammostola rosea) spiderlings were placed on a white surface and escape responses were evoked by directing a 980nm laser at each of the spider's 8 legs. Escape movements were recorded with a high-speed digital camera. Marks applied to both the cephalothorax and abdomen were automatically tracked measure spider location and orientation. Results revealed that responses consisted of five stages, characterized by sequential: 1) limb movement, 2) small (~1 mm) movement away from the stimulus, 3) rotation of body away from the stimulus, 4) forward locomotion and 5) a broad curve in locomotion. Quantification of the direction of the stage 2 responses and stage 3 rotations revealed that the directions/rotations of response were dependent on which leg was stimulated. While stage 2 responses were directly away from the stimulus, stage 3-4 rotation and locomotion was in a direction midway between directly away from the stimulus and in the direction the spider was pointing. Speed of response was independent of stimulus location. These results demonstrate that Chilean rose tarantula spiderlings exhibit complex, reproducible escape responses that are amenable to further quantitative analysis. Funded by NSF.

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NEUROBEHAVIORAL AND ENDOCRINE CHANGES IN A RAT MODEL OF MAMMARY ADENOCARCINOMA: EFFECTS OF MELATONIN

Melatonin, a hormone produced in the pineal gland at night, has been shown to attenuate tumor progression, as well as have anxiolytic and antidepressant properties. This study investigated if mammary adenocarcinoma can induce changes in anxiety-like and depression-like behaviors in a tumor-bearing rat model, and to assess if melatonin could attenuate either tumor progression or behavioral changes in this model. Female Fisher 344 rats were injected subcutaneously in the mammary pad with either serum free medium or $1.0x10^6$ MTLn3 rat adenocarcinoma cells for tumor growth. Once tumor growth occurred palpable tumor size was recorded daily. Melatonin was administered in the drinking water at a target dose of 4 mg/kg/day beginning 2 days prior to MtLn3 cell injections. For the assessment of anxiety-like behaviors, animals were tested on the elevated plus maze 19-20 days following control or MtLn3 cell injections. Two days later, immobility, climbing and swimming behaviors were evaluated using the Porsolt forced swim test. Circulating levels of melatonin, corticosterone, estradiol and progesterone following the stress were also determined. Circulating and brain melatonin levels were higher, even in daylight hours, in animals treated with melatonin compared with controls (F-19, $P<0.0001$), although tumor-bearing rats showed significantly reduced levels of melatonin compared to controls ($F=6.3$, $P<0.02$; melatonin levels were 69±12, 733±181 and 246±56 pg/ml in water controls, melatonin-treated non-tumor, melatonin-tumor bearing groups, respectively). Tumor-bearing rats showed decreased open arm time in the plus maze, indicative of increased anxiety-like behaviors ($P<0.05$), and also a decrease in circulating progesterone levels (F-4, $P=0.05$). Melatonin failed to significantly attenuate tumor progression or behavioral changes, which could be related to decreased circulating melatonin levels in tumor-bearing rats.

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THE ANOREXIGENIC EFFECTS OF XENIN ARE MEDIATED DIRECTLY VIA THE VENTROMEDIAL HYPOTHALAMUS IN CHICKS

Previously we demonstrated that intracerebroventricular (ICV) and peripheral injection of xenin, a 25 residue neuropeptide, caused anorexigenic effects in 4-day post hatch chicks. We also determined that both routes of administration caused increased neuronal activation in the ventromedial hypothalamus (VMH), the classical satiety center. Thus, we designed the present experiment to determine if xenin directly causes VMH activation. Chicks, 16 days post hatch, were stereotaxically implanted with a guide cannula positioned 0.5 mm superior to the left VMH. Two days after recovery 180 min fasted chicks randomly received 1.5 or 0 ?g of xenin via the guide cannula. Direct injection of xenin onto the VMH caused decreased feed intake (9.1±1. vs. 016.4±2.2 g, $P < 0.05$), with no effect on water. To determine where the effect was mediated we quantified c-Fos expression in the VMH and lateral hypothalamus (LH) 60 min after injection.

Chicks in the xenin group had higher (42.8±4.7, P < 0.05) VMH neuronal activation than did controls (23.4±4.3). LH activation was not effected. These results demonstrate that the anorexigenic effects of xenin are mediated directly at the VMH in chicks.

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GAMMA OSCILLATION INCREASES DURING RANDOMIZED COGNITIVE TASKS

Gamma electroencephalogram (EEG) oscillations (30-100 Hz) are involved in perception and cognition and correlate with cognitive task execution. Alpha oscillation, on the other hand, represents a non-arousal state associated with awake resting. We tested the presence of Alpha as a within-subject control on the induction of Gamma power during randomized cognitive tasks presentations. EEG was recorded from 30 participants. Three scalp electrode sites (Fz, P3 & P4) referenced to ears and nose were used. Participants gave informed consent and were guided trough six cognitive tasks (film watching, story reading, learning a word list, subtraction task, music listening, and recalling the learned words). Resting with eyes closed between tasks (~ 30 s) was used as a control situation. Preliminary analysis of data shows an increase of Gamma oscillations on the posterior sites (P3 & P4) for the following tasks: film watching, story reading, subtraction task and learning words!, with the latter task also showing Gamma increases in the frontal site. This study highlights the association between Gamma power and cognitive tasks and shows that Gamma oscillation induction is independent of Alpha rhythms.

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LONG-TERM POTENTIATION IN THE CROSSED TEMPORODENTATE PATHWAY FOLLOWING UNILATERAL ENTORHINAL CORTEX LESIONS IN THE RAT

Following unilateral denervation of the dentate gyrus (DG) by lesion of the entorhinal cortex (EC), rats lose their ability to complete a learned spatial alternation task. About 8 to 12 days after the entorhinal injury, the rats regain the ability to alternate on the spatial task. The time course of recovery parallels that for sprouting by the crossed temporodentate pathway (CTD), which arises from the intact entorhinal cortex and terminates in the denervated DG. The physiological mechanism for behavioral recovery has not been determined, but the emergence of the CTD's ability to support long-term potentiation (LTP) is a possibility. In this study, we assessed the CTD's ability to sustain LTP 8 days following one-stage or progressive unilateral electrolytic lesion of the EC. Using a 15% increase in maximum amplitude or maximum slope of evoked potentials as the criterion for LTP, we observed that neither the one-stage nor the progressive group exhibited the capacity for LTP. This finding provides evidence that LTP may not be responsible for recovery of spatial alternation behavior following unilateral lesion of the EC in rats.

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RELATIONSHIPS BETWEEN SERUM TOTAL CHOLESTEROL LEVELS AND COGNITIVE FUNCTION

Previous research regarding the relationship between total serum cholesterol (TC) and cognition in humans has provided conflicting and inconclusive results, with some studies finding a negative relationship between cholesterol levels and cognitive performance, and others finding a positive relation between the two. Few studies have focused on a non-elderly population, and stress has not been considered despite its relevance to both serum cholesterol levels and cognitive performance. This study aimed to investigate the potential relationship between serum TC levels and cognitive functioning in a non-elderly population while controlling for life stress. Our sample consisted of faculty and staff from a private university who participated in an employee-provided health screening. At the time of this screening, measurements of serum TC as well as other aspects of physical health were obtained. After completing the health screening, planning abilities and sustained! attention/psychomotor speed were assessed in each participant using the Tower of London (TOL) and the Digit Vigilance Test (DVT) respectively. The Life Stressors and Social Resources Inventory-Adult Form (LISRES-A) was administered as a standardized measure of life stress. The results of these neurocognitive assessments were then correlated with serum TC, and a covariate-adjusted analysis was performed to examine potential relationships between these variables while controlling for life stress and other selected socio-demographic variables. The results of these analyses suggest that cognitive function in humans may be significantly altered by differing serum levels of TC.

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CHARACTERIZING DROSOPHILA PALMIOTYL-PROTEIN THIOESTERASE 1'S ROLE IN THE ENDO-LYSOSOMAL PATHWAY

Infantile Neuronal Ceroid Lipofuscinosis is caused by mutations in the cln1 gene which encodes palmitoyl-protein thioesterase 1 (Ppt1), suggesting that there is an important role for the regulation of palmitoylation in normal neuronal

function. Loss of Ppt1 function in patients produces granular osmiophilic deposits (GRODS) in all cells and massive neurodegeneration. Consistent with an important role for PPT1 in neurons, the protein is found with synaptic vesicles and synaptosomes in neuronal cell culture. Recently, we reported the results of a gain-of-function modifier screen in which we used ~2000 EP and EY transgenic overexpression lines to suppress or enhance the degeneration produced by Drosophila Ppt1 overexpression. The 20 modifier genes we identified link Ppt1 function to endocytosis, synaptic development and synaptic function. We are pursuing the link to endocytosis by examining Ppt1 mutant flies for defects in the endo-lysosomal pathway. Our preliminary experiments with larval garland cells suggest that mutant flies have a defect in fluid-phase endocytosis. We will present these results and our plans for future characterization of the endocytosis phenotype.

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SEIZURE INDUCED NEURODEGENERATION IMPAIRS SPATIAL MEMORY IN TEMPORAL LOBE EPILEPSY

Temporal lobe epilepsy (TLE) is a common neurological disorder with a prolonged and intractable course. Cognitive function among patients with TLE is typically characterized by significant memory impairment. In these patients the cumulative effects of repeated spontaneous seizures can worsen cognitive impairment. The neurobiological mechanism underlying the progression in cognitive impairment is not well understood. To address this issue we have examined memory function and neurodegeneration in brain during TLE in a rat model. Chronic TLE was induced in eleven adult male Sprague-Dawley rats by injection of pilocarpine. Ten animals were treated with saline and used as controls. All animals treated with pilocarpine developed chronic epilepsy. Ten days after pilocarpine or saline treatment spatial memory and social recognition memory were assessed using Morris water maze and social recognition tests. At this time structural changes in the brain were also evaluated with T2 weighted magnetic resonance images (MRI) from six animals in each group. To assess progressive changes these measures were again obtained 12 weeks post-treatment. Compared with controls, pilocarpine treated rats exhibited impaired performance on the Morris water maze at 10 days post-treatment. This impairment was markedly greater at 12 weeks post-treatment. In contrast, there was a decline in performance in social recognition tests that did not become worse over time. MRI scans revealed increased signal intensity indicative of damage in limbic structures at 10 days, with an increase in damage in hippocampus and thalamus at 12 weeks post-treatment. These results demonstrate progressive impairment in performance in a spatial task, but not social recognition memory during TLE. Furthermore, they suggest that the increase in structural damage in hippocampus and potentially in the thalamus in these animals contributes to this impairment.

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DIFFERENTIAL EFFECTS OF α-MSH ON SOCIAL BEHAVIOR IN MICE DEPENDING UPON THE MCR1 RECEPTOR

Introduction: Previous research indicates that α-MSH (melanocyte-stimulating hormone) modulates pain in a sex dependent manner (Mogil et. al., 2003). The mechanism of action for this sex difference involves the melanocortin-1 receptor, which known to be richly present in the periaqueductal gray.
Goals: Because this area is implicated in sexual and social drives, we investigated the role of this peptide-receptor interaction in opposite-sex social behavior. We further explored this by studying the effect of female estrous cycles on social behavior. Speculation as to the effect of α-MSH in the absence of the melanocortin-1 receptor led to our further testing on MCR1 mice.
Methods: One hundred and thirty adult, naïve Swiss Webster (outbred) and melanocortin-1 receptor knockout (MCR1) mice were randomly assigned a partner of the same strain, and opposite sex to one of eight experimental conditions, which were varied based on drug type (saline or α-MSH) and estrous status. Based on vaginal appearance, females were verified as proestrus/estrus (receptive) or metestrus/diestrus (non-receptive). Twenty minutes after intracerebroventricular (ICV) injections of saline or α-MSH, the pair was placed in a social interaction box for 10 minutes, in which the following behaviors were quantified: locomotor activity (line crosses), flees, tailfollows, anogenital investigation, frontal investigations, rears, self-groomings, mountings, initiate contacts, and squeaks.
Results: Data shows that Swiss Webster females significantly decrease tailfollowing when in estrus. Swiss Webster females on α-MSH paired with Swiss Webster males on saline significantly decrease tailfollowing. MCR1 females significantly flee more and tailfollow less with saline males. A-MSH or estrous females paired with α-MSH males tailfollow significantly more; thus, α-MSH appears to mimic estrous. MCR1 saline males flee significantly less with estrous females than their α-MSH counterparts. α-MSH males also tailfollow less.
Conclusion: Data suggests that α-MSH exhibits a higher potency in the MCR1 strain, and that it may mimic estrous in female mice.

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VAGOTOMY VERIFICATION UTILIZING IMMUNOHISTOCHEMISTRY AND LIGHT MICROSCOPY

In the past, verification of the surgical procedure, subdiaphragmatic vagotomy has been conducted by measuring postmortem stomach weights or by the fluorescent retrograde tracer, Fluorogold. Both methods come with obstacles. Measuring the postmortem stomach weight increases probability of investigator error due to variability of rodent stomach anatomy. Fluorescent microscopy tends to be expensive and requires a high level of expertise to master. To circumvent these challenges a new approach to vagotomy verification was sought. Fluorochrome, an antibody to the retrograde tracer Fluorogold, has been used in immunohistochemistry studies for neuronal counts where long-lasting staining is preferred. Perfusions and brain sectioning are now common practices within many research labs where investigation of brain mechanism plays a central role. Enzyme-linked immunoabsorbent assay (ELISA) is readily available and relatively easy for undergraduate researchers to learn. In our study, Fluorogold tracer was applied to the transected nerve during vagotomy surgery. Following a recovery period and after behavioral testing verification of the surgery was obtained by using the florochrome antibody as part of an ELISA procedure. The stained antibody allowed for a direct measure of vagotomy completeness that could be verified by light microscopy. By utilizing the retrograde tracer Fluorogold in conjunction with Immunohistochemistry and the antibody, Fluorochrome, an easy, efficient and relatively inexpensive method to verify vagotomy was established.