

# The 26th Annual Winter Neuropeptide Conference



January 29<sup>th</sup>-February 1<sup>st</sup>, 2005  
Breckenridge, Colorado

The International Neuropeptide Society is Pleased to Announce The  
26<sup>th</sup> Annual Winter Neuropeptide Conference  
*Beaver Run Resort and Conference Center*  
*January 29th-February 1st, 2005*

**Treasurer and Facilities Chair**

Curt A. Sandman  
University of Cal., Irvine  
Dept. of Psychiatry  
2501 Harbor Boulevard, 7A  
Costa Mesa, CA 92626  
714-957-5435  
FAX: 714-957-5354  
casandma@uci.edu

**Program Chair**

Francis Flynn  
University of Wyoming  
Dept. of Zoology & Physiology  
Box 3166, University Station  
Laramie, WY 82071  
307-766-6446  
FAX: 307-766-2926  
flynn@uwyo.edu

**Steering Committee**

Doug Brenneman  
Catherine Spong  
Joanna Hill  
Roger Smith  
Illana Gozes  
Fleur Strand  
John Quinn  
Cecelia Sladek  
Bibie Chronwall  
Tom Davis

**Conference Coordinator**

Deb Edwards  
Conference Coordinator  
P.O. Box 7518  
Breckenridge, CO 80424  
Phone: 970-453-5970  
Fax: 970-453-1423  
tsfdirector@summitfoundation.org

WELCOME TO THE ANNUAL WINTER NEUROPEPTIDE  
CONFERENCE.

We are pleased to be hosts for this, the twenty-sixth year of the conference.

We hope that your meetings will be profitable and your stay in Breckenridge relaxing and enjoyable. If we can be of any assistance, just let us know.

Cordially,

*Deb Edwards*

Deb Edwards  
Conference Coordinator



❄️ Sponsors

The Winter Neuropeptide Conference would like to acknowledge and thank the following companies for the financial support of the 2005 conference.

Johnson & Johnson Pharmaceutical Research & Development

National Institute of Health

*The Winter Neuropeptide Conference is an affiliate of the International Neuropeptide Society.*

\*\* Oldest Neuropeptide Conference in North America.

 Acknowledgements

Bill Flynn– Program Chair  
Curt Sandman-Treasurer & Facilities Chair  
Doug Brennan– Steering Committee  
Catherine Spong-Steering Committee  
Jonanna Hill-Steering Committee  
Roger Smith– Steering Committee  
Illana Gozes– Steering Committee  
Fleur Strand– Steering Committee  
John Quinn– Steering Committee  
Celia Sladek– Steering Committee  
Bibie Cronwall– Steering Committee  
Tom Davis– Steering Committee  
Bernard Westerop, Elsevier Science  
Abba Kastin– Editor, Peptides  
The International Neuropeptide Society  
Debra Edwards– Conference Coordinator  
Jody Wagner– Beaver Run Resort  
Lawson Pedder– Beaver Run Resort  
Beaver Run Resort  
Colorado Mountain Express;  
Hearthstone Restaurant  
Brekenridge Ski Resort  
Johnson & Jonshon  
National Institute of Health  
University of Wyoming  
University of California, Irvine  
Summit Foundation

## Schedule-at-a-Glance

Registration – 1<sup>st</sup> Floor South Foyer of the Conference Center

All symposia meetings will be held in Peak 17, 1<sup>st</sup> Floor Conference Center

Poster Session & Dinner Buffet will be held in Peaks 15 & 16, 1<sup>st</sup> Floor Conference Center

### Saturday, January 29

2 – 8 PM	Registration/Check-in Desk Open
3:45 PM	Welcome and Opening Remarks
4 – 6 PM	Symposia Meeting (Beverages & Light Refreshments Provided)
6 – 7:30 PM	Symposia Meeting (Beverages Provided)
7:30 – 9:30 PM	Poster Session & Dinner Buffet

### Sunday, January 30

3 – 10 PM	Registration/Check-in Desk Open
4 – 6 PM	Symposia Meeting (Beverages & Light Refreshments Provided)
6 – 8 PM	Dinner Break (on your own)
8 – 10 PM	Symposia Meeting (Beverages Provided)

### Monday, January 31

4 – 6 PM	Symposia Meeting (Beverages & Light Refreshments Provided)
6 – 8 PM	Dinner Break (on your own)
7 – 10 PM	Registration/Check-in Desk Open
8 – 10 PM	Symposia Meeting (Beverages & Light Desserts Provided)

### Tuesday, February 1

3:30 – 6 PM	Registration/Check-in Desk Open
4 – 6 PM	New Investigator Session (Beverages & Light Refreshments Provided)

## Saturday, January 29, 2005

**3:45 PM:** Welcome and Opening remarks.

**4:00- 6:00 PM: Volume Transmission: Hardcore Evidence?** Donal Skinner, University of Wyoming, Laramie, WY.

- **Sex, GnRH and Cerebrospinal Fluid.** Donal Skinner, University of Wyoming, Laramie, WY.
- **Does cerebrospinal fluid melatonin transduce photoperiod in the brain?** Benoit Malpoux, Institut National de la Recherche Agronomique, France.
- **Diffusible signals in the circadian and GnRH systems.** Michael Lehman, University of Cincinnati, Cincinnati, OH.

**6:00 – 7:30 PM: Epilepsy** Michael Kubek. Indiana University School of Medicine, Indianapolis, IN.

- **New insights into the function and delivery of TRH to specific temporal lobe targets.** Michael Kubek. Indiana University School of Medicine, Indianapolis, IN.
- **Seizure susceptibility in NPY protein and receptor knockout mice.** Harlan Shannon, Neuroscience Division, Eli Lilly & Company, Indianapolis, IN.
- **Galanin is a potent anticonvulsant.** Claude Wasterlain, UCLA Geffen School of Medicine, Los Angeles, CA.

***Dinner buffet provided (Posters during dinner)***

## Sunday, January 30, 2005

### **4:00-6:00 PM: Visceral Afferent Neuropeptide Pathways Controlling Ingestion.**

Gaylen Edwards, University of Georgia, Athens, GA.

- **Responding to Mixed Messages: Interaction of peptidergic, mechanoreceptive and nutritive signals in the vagal afferent control of food intake.** Robert Ritter, Washington State University, Pullman, WA.
- **Gut feelings: Cellular mechanisms for detection and integration of gastrointestinal and metabolic signals by primary vagal afferents.** Steve Simasko, Washington State University, Pullman, WA.
- **Feeding Highs: Hindbrain Actions of Cannabinoids and Potential Interactions with Peptidergic Signals.** Gaylen Edwards, University of Georgia, Athens, GA.
- **Evidence that paraventricular nucleus oxytocin neurons link hypothalamic leptin action to caudal brain stem nuclei controlling meal size.** J. E. Blevins, VA Medical Center, Seattle, WA.

### **8:00- 10:00 PM: Biotechnology.** Illana Gozes, Tel Aviv University, Tel Aviv Israel

- **Milestones and Millstones; from concept to commercialization.** James Miller, Chairman, Allon Therapeutics, Inc Vancouver, BC, Canada.
- **Peptides: toward drug development.** Illana Gozes, Tel Aviv University, Tel Aviv Israel and Allon Therapeutics, Inc. Vancouver, BC, Canada.
- **What big pharma looks for in evaluating preclinical technology.** Douglas Breneman, Johnson & Johnson Pharmaceutical Research & Development, Spring House, PA.

## Monday, January 31

**4:00-6:00 PM: Mechanisms modulating neuropeptide plasticity in the CNS.** John Quinn, University of Liverpool, UK.

- **Cytokine modulation of neuronal gene expression.** Andy Russo, University of Iowa, Iowa City, IA.
- **Does NO have a say in Neuropeptides regulation?** Thippeswamy Thimmasettappa, University of Liverpool, Liverpool, UK.
- **Neuropeptide Y and Hippocampal Neurogenesis.** Liam Gray, University of Southampton, UK.
- **ADNP expression and function: lessons from knockout mice.** Illana Gozes, Tel Aviv University, Tel Aviv Israel and Allon Therapeutics, Inc. Vancouver, Canada.

**8:00-10:00 PM: Developmental effects of CRH.** Curt Sandman, University of California at Irvine, Irvine, CA.

- **Activation of the HPA and placental axis influences birth outcome and the human fetus.** Curt Sandman, University of California, Irvine, CA.
- **Long-term, progressive hippocampal cell loss and dysfunction induced by early-life administration of corticotropin-releasing hormone reproduce the effects of early-life stress.** Kristen Brunson, University of California, Irvine, CA.
- **Evolution of diversity of corticotropin-releasing factor signaling.** Robert J. Denver, University of Michigan, Ann Arbor, MI.

**Tuesday, February 1, 2005**

**4:00-6:00 PM: Young Investigator Symposium**

**A SPECIAL COMPETITION FOR YOUNG INVESTIGATORS** (less than 5 years postdoctoral) will be held, with the winners receiving travel funds, a certificate, and the opportunity to present their work in a special Young Investigator Symposium. This program is supported by Johnson & Johnson and the NICHD.

**7:00 PM: *Dinner at Hearthstone Restaurant*** (additional fee and registration required)

**EVIDENCE THAT PARAVENTRICULAR NUCLEUS OXYTOCIN NEURONS LINK HYPOTHALAMIC LEPTIN ACTION TO CAUDAL BRAIN STEM NUCLEI CONTROLLING MEAL**

**SIZE.** J.E. Blevins<sup>1,3</sup>, G.J. Morton<sup>3</sup>, D.L. Williams<sup>3</sup>, D.W. Caldwell<sup>1</sup>, M.W. Schwartz<sup>3</sup>, and D.G. Baskin<sup>1,2,3</sup>. <sup>1</sup>Metab., VA Medical center, Seattle, WA, USA ; <sup>2</sup>Biological Structure, Univ. Washington, Seattle, WA, USA; <sup>3</sup>Medicine, Univ. Washington, Seattle, WA, USA

Adiposity signals such as leptin are thought to reduce food intake in part by enhancing the response to satiety signals such as cholecystokinin (CCK). We and others hypothesize that leptin activates parvocellular paraventricular nucleus (pPVN) neurons, including those that contain oxytocin (OXY), which project to the nucleus of the solitary tract. To test this hypothesis, we investigated the ability of leptin to activate OXY neurons in the pPVN and determined whether the ability of leptin to reduce food intake requires OXY. We found that third ventricle (3V) administration of leptin at a dose that inhibits feeding increased the number of pPVN OXY neurons that showed positive immunostaining for Fos by two-fold ( $p < 0.05$ ). A subset of these leptin-activated pPVN neurons were also labeled after injection of a retrograde tracer (cholera toxin subunit B) into the region of the NTS, demonstrating the existence of a leptin-sensitive oxytocinergic projection from the pPVN to the hindbrain. Moreover, we found that 3V administration of a subthreshold dose of an OXY receptor antagonist [D-(CH<sub>2</sub>)<sub>5</sub>, Tyr(Me)<sub>2</sub>, Orn<sup>8</sup>]-vasotocin (OVT) attenuated the ability of leptin to reduce food intake over 4-h ( $p < 0.05$ ). To determine whether OXY contributes to the ability of leptin to enhance CCK-induced Fos in the medial NTS (mNTS), we administered OVT in the 3V before 3V leptin and intraperitoneal (i.p.) CCK-8 administration. OVT resulted in a significant 37% decrease ( $p < 0.05$ ) in the ability of leptin to enhance CCK-induced Fos in the mNTS. In a separate study, fourth ventricular (4V) administration of OVT stimulated 2-h food intake by 43% ( $p < 0.01$ ), whereas 3V injection of the same dose was ineffective, supporting the suggestion that endogenous OXY release within the hindbrain tonically inhibits food intake. Furthermore, CCK-induced satiety was significantly attenuated by pre-treatment with a dose of 4V OVT that was subthreshold for feeding effects when given alone, consistent with the idea that OXY action in the hindbrain contributes to the ability of CCK to reduce food intake. Based on the findings, we suggest that leptin affects food intake in part through activating an oxytocinergic pPVN-to-NTS projection that increases the hindbrain's sensitivity to satiety signals. Among several neuropeptide systems considered to be downstream mediators of leptin's effects, the melanocortin system has received a great deal of attention. We have recently demonstrated that 3V administration of the melanocortin agonist (melanotan II) enhanced the ability of CCK to elicit Fos in the NTS and inhibit food intake. These data make plausible the suggestion that the ability of leptin to interact with CCK is mediated in part through activation of melanocortin neurons. Taken together, these findings support the hypothesis that leptin enhances the hindbrain response to satiety signals by activating a descending oxytocinergic projection from the pPVN to the NTS.

**FEMTOMOLAR MEDIATION OF MICROGLIA-DERIVED OXIDATIVE STRESS AND DOPAMINERGIC NEURON SURVIVAL: SUBSTANCE P VS. DYNORPHIN.** M.L. Block, G. Li, L. Qin, Z. Pei, X. Wu, B. Wilson, J.S. Hong. Neuropharmacology Section, Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709

Inflammation is recognized to contribute to the pathogenesis of Parkinsons' disease (PD), where unregulated microglial activation is implicated as a pivotal factor. Currently, the homeostatic mechanisms controlling microglial function in the substantia nigra (SN) are unknown. In the following study, we address the novel possibility that peptides endogenous to the SN, Substance P (SP) and Dynorphin A (DynA), work at femtomolar (fM) concentrations as opposing mediators of microglial activation and consequent DA neurotoxicity. To begin testing this hypothesis, neuron-glia cultures were exposed to fM concentrations of SP, resulting in a decrease in both the DA uptake and the number of DA neurons. The selectivity of fM SP for DA neurons was demonstrated by a lack of SP effect on both the uptake of other neurotransmitters (GABA) and the over-all neuron cell number in neuron-glia cultures. The critical role of microglia was evident by the failure of neuron-enriched cultures to exhibit fM SP DA neurotoxicity, where DA neuron death was reinstated with the addition of microglia back to neuron-enriched cultures. The addition of fM SP to enriched microglia cultures elicited both intracellular reactive oxygen species (ROS) and superoxide (SO), supporting that fM SP activates microglia. Further, neuron-glia cultures from NADPH oxidase deficient mice, which are unable to produce extracellular SO, were insensitive to fM SP-induced DA neurotoxicity, emphasizing the critical role of microglial derived ROS in fM SP neurotoxicity. Here, we are the first to identify a fM neurotoxic peptide, SP, which is selectively toxic to DA neurons through microglia-generated oxidative insult. DynA (fM) has been previously established to be both anti-inflammatory and neuroprotective through the inhibition of microglial NADPH oxidase. To test which peptide, SP or DynA, would prevail in the determination of DA neuron survival, neuron-glia cultures were exposed to both fM-acting peptides. DynA ( $10^{-14}$ M) inhibited both SP ( $10^{-13}$ M)-induced DA neurotoxicity and microglial ROS production. In summary, we demonstrate a tightly regulated mechanism governing microglia-derived oxidative stress, where the fM neuropeptide balance of Dyn and SP is critical to DA neuron survival, offering both insight into the potential etiology of PD and hope for the development of innovative therapeutic compounds.

**LONG-TERM, PROGRESSIVE HIPPOCAMPAL CELL LOSS AND DYSFUNCTION INDUCED BY EARLY-LIFE ADMINISTRATION OF CORTICOTROPIN-RELEASING HORMONE REPRODUCE THE EFFECTS OF EARLY-LIFE STRESS.** Brunson KL, Fenoglio K, Bender R, Chen Y, Baram TZ. Department of Anatomy/Neurobiology, University of California, Irvine, CA 92697-4475, USA.

Stress early in postnatal life may result in long-term memory deficits and selective loss of hippocampal neurons. The mechanisms involved are poorly understood, but they may involve molecules and processes in the immature limbic system that are activated by stressful challenges. We report that administration of corticotropin-releasing hormone (CRH), the key limbic stress modulator, to the brains of immature rats reproduced the consequences of early-life stress, reducing memory functions throughout life. These deficits were associated with progressive loss of hippocampal CA3 neurons, mossy fiber sprouting and chronic up-regulation of hippocampal CRH expression. In addition, administration of CRH antagonist attenuated memory deficits induced by early-life stress. These findings indicate a critical role for CRH in the mechanisms underlying the long-term effects of early-life stress on hippocampal integrity and function.

**UROCORTIN I AND UROCORTIN III REDUCE MATERNAL AGGRESSION AND INCREASE FOS-IR IN THE LATERAL SEPTUM AND BED NUCLEUS OF THE STRIA TERMINALIS.**

Kimberly L. D'Anna, University of Wisconsin

Female rodents fiercely defend their offspring during lactation while exhibiting decreased indices of fear and anxiety. To understand the inverse relationship between fear and anxiety and aggression, we examined the effects of Urocortin I (Ucn I) and Urocortin III (Ucn III), two anxiogenic peptides, on the production of maternal aggression. Both Ucn I and Ucn III are closely related to corticotropin-releasing factor (CRF), another anxiogenic peptide that recently has been shown to inhibit maternal aggression via intracerebroventricular (icv) injections. Both icv Ucn I (0.2, but not 0.02  $\mu\text{g}$ ) and Ucn III (0.5, but not 0.2 or 0.02  $\mu\text{g}$ ) reduced overall levels of aggression, but not other maternal behaviors, such as pup retrieval. Icv injections of both Ucn I (0.2  $\mu\text{g}$ ) and Ucn III (0.5  $\mu\text{g}$ ) also induced significantly higher levels of Fos immunoreactivity (Fos-IR) in two common brain regions, lateral septum (LS) and bed nucleus of the stria terminalis (BNST), relative to vehicle injections. Both brain regions have previously been implicated in the production of maternal aggression. Further, icv CRF, at a dose that inhibits maternal aggression (0.2  $\mu\text{g}$ ), also elevates Fos in these regions. These findings suggest that Ucn I and CRF are equally effective in inhibiting maternal aggression and that Ucn III is a less effective inhibitor. Because all three peptides affect Fos-IR within LS and BNST while inhibiting aggression, it is possible that these brain regions are critical sites for the negative regulation of maternal aggression. These findings are consistent with the idea that the neurotransmission of CRF and related peptides, Ucn I and Ucn III, is reduced during lactation and that the concomitant decreases in fear and anxiety are a critical step in the normal production of maternal aggression. Supported by NIMH Grant R01MH066086 to S.C.G.

**EVOLUTION OF DIVERSITY OF CORTICOTROPIN-RELEASING FACTOR SIGNALING.**

Robert J. Denver<sup>1,2</sup>, Graham C. Boorse<sup>2</sup>, Frank M. Dautzenberg<sup>3</sup>, and Erica J. Crespi<sup>1</sup>. <sup>1</sup>Department of Molecular, Cellular and Developmental Biology and <sup>2</sup>Department of Ecology and Evolutionary Biology, The University of Michigan, Ann Arbor, MI 48109-1048 USA and <sup>3</sup>Johnson & Johnson, Research & Development, CNS Research, Turnhoutseweg 30, Building 162, Room 206 B-2340 Beerse, Belgium.

The vertebrate stress neurohormone corticotropin-releasing factor (CRF) plays critical roles in development, physiology and behavior. CRF is known to control the timing of life history transitions such as amphibian metamorphosis and mammalian birth. Vertebrates have several CRF-like peptides that bind to and activate two G protein-coupled receptors (CRF<sub>1</sub> and CRF<sub>2</sub>). Mammals have four CRF-like peptides: CRF, urocortin 1 (UCN1), UCN2 and UCN3. In teleost fishes, CRF, urotensin I (a fish ortholog of mammalian UCN1) and UCN3 have been identified. In amphibians, CRFs have been isolated from five anuran species, but sauvagine is the only urotensin I/UCN-like peptide known. We isolated cDNAs for two urocortins, UCN1 and UCN3, from the South African clawed frog *Xenopus laevis*. Also, by analyzing genomic databases we discovered novel CRF-like peptides in pufferfish and chicken. Molecular phylogenetic analysis of all known vertebrate CRF-like peptides and those identified by us supports the existence of four paralogous lineages in tetrapods that likely arose before the radiation of the teleost fishes. The UCN2 lineage is the least resolved. We synthesized the *X. laevis* urocortins and studied their ligand-binder pharmacology and physiology. *X. laevis* CRF<sub>1</sub> and CRF-binding protein (CRF-BP) had higher affinity for CRF than for UCN1; whereas, CRF<sub>2</sub> bound UCN1 with similar affinity to CRF. Similar to mammals, *X. laevis* UCN3 is a selective ligand for CRF<sub>2</sub> and is not bound by the CRF-BP. ICV injections of both UCN1 and UCN3 inhibited food intake in juvenile *X. laevis*, thus supporting a role for the CRF<sub>2</sub> in the appetite-suppressive effects of CRF-like peptides in amphibians as has been shown in mammals. Also, while CRF<sub>1</sub> mediates the actions of CRF-like peptides on ACTH, the CRF<sub>2</sub> mediates actions on TSH secretion (supported by NSF grants IBN9974672 and IBN0235401).

**FEEDING HIGHS: HINDBRAIN ACTIONS OF CANNABINOIDS AND POTENTIAL INTERACTIONS WITH PEPTIDERGIC SIGNALS.** Gaylen L. Edwards and Cheryl C. Miller. Dept. of Physiol. & Pharmacol., Univ. of Georgia, Athens, GA 30605 and Dept. of Diet. & Nutr. , Univ. of Kansas Medical Center, Kansas City, KS 66160.

The role of the hindbrain in the control of ingestive behavior is now recognized to include motivational aspects of food intake. Lesions placed in the dorsal vagal complex (DVC) produce dramatic changes in energy balance and food intake. Altered behaviors in lesioned rats include marked increases in the ingestion of highly palatable foods and increased operant responding for sweet solutions. These findings suggest increased motivation to obtain highly palatable substances by rats with hindbrain lesions. One family of substances that have come to the forefront as important molecules in the control of motivation-induced ingestive behavior is the cannabinoids. Most research has focused on forebrain systems classically associated with motivation and reward. However, a recent report from our group (Physiol. Behav. 80: 611-616, 2004) indicates that cannabinoids injected into the hindbrain facilitate intake of highly palatable foods at much lower doses than those used in the forebrain. A potential site for hindbrain actions of cannabinoids is the nucleus of the solitary tract. Cannabinoid receptors are abundant in this region. Moreover, primary gustatory and gastrointestinal afferent nerves terminate in this nucleus. Thus, cannabinoids could interact with these afferent systems to suppress signals arising in the gut that inhibit food intake such as cholecystokinin (CCK). As it is reported that cannabinoids act at presynaptic sites to inhibit transmission in areas such as the hippocampus (Eur. J. Pharmacol. 469: 47-55, 2003), it is possible that cannabinoids act presynaptically on visceral afferents to suppress activity in these neurons. Since these afferents are activated by gastrointestinal satiety signals such as CCK, discussion will focus on potential sites of action of cannabinoids on visceral afferent projections that could contribute to the facilitation of food intake by cannabinoids.

**OPIOID RECEPTOR INVOLVEMENT IN FOOD DEPRIVATION-INDUCED FEEDING: EVALUATION OF SELECTIVE ANTAGONISTS AND ANTISENSE OLIGODEOXYNUCLEOTIDE PROBE EFFECTS IN MICE AND RATS.** Maria M Hadjimarkou<sup>1</sup>, Amreeta Singh<sup>1</sup>, Yakov Kandov<sup>1</sup>, Yuriy Israel<sup>1</sup>, Ying-Xian Pan<sup>2</sup>, Grace C. Rossi<sup>3</sup>, Gavril W. Pasternak<sup>2</sup>, Richard J. Bodnar<sup>1</sup>. <sup>1</sup>Queens College, CUNY; <sup>2</sup>Memorial Sloan-Kettering Cancer Center; <sup>3</sup>Long Island University, NY.

Central administration of general and selective opioid receptor subtype antagonists in the rat has revealed a substantial role for  $\mu$ , a moderate role for  $\kappa$  and a minimal role for  $\delta$  receptors in the mediation of deprivation-induced feeding. Antisense probes directed against the KOP, NOP and DOP genes in rats result in reductions similar to  $\kappa$  and  $\delta$  antagonists, whereas antisense probes directed against the MOP gene produced modest reductions relative to  $\mu$  antagonists, suggesting that isoforms of the MOP gene may mediate deprivation-induced feeding. Since these isoforms were initially identified in mice, the present study compared the effects of general and selective opioid receptor antagonists on deprivation-induced feeding in rats and mice, and antisense probes directed against exons of the MOP, DOP, KOP and NOP genes on deprivation-induced feeding in the mouse. Food-deprived (12 and 24 h) rats and mice displayed similar profiles of reductions in deprivation-induced feeding following general,  $\mu$  and  $\kappa$  opioid antagonists. In contrast, mice, but not rats displayed reductions in deprivation-induced intake following  $\delta$  antagonism as well as DOP antisense probes, suggesting a species-specific role for the  $\delta$  receptor. Antisense probes directed against the KOP and NOP genes also reduced deprivation-induced intake in mice in a manner similar to  $\kappa$  antagonism. However, the significant reductions in deprivation-induced feeding following antisense probes directed against either exons 2, 4, 7, 8 or 13 of the MOP gene were modest compared to  $\mu$  antagonism, suggesting a role for multiple  $\mu$ -mediated mechanisms.

**AGONIST AND HYPERTONIC SALINE-INDUCED TRAFFICKING OF NK3 RECEPTOR ON VASOPRESSIN NEURONS.** Gwendolen E. Haley and Francis W. Flynn, Neuroscience Program, University of Wyoming, Laramie, WY 82071

Studies have shown that the neurokinin 3 receptor (NK3-R) is colocalized with vasopressin neurons within the paraventricular nucleus of the hypothalamus (PVN). The functional role of NK3-R in vasopressin release under physiological challenges is not known. Injections of selective NK3R agonist, such as senktide, stimulate a systemic vasopressin release and induce c-fos expression in magnocellular vasopressinergic neurons. It is unknown if the c-Fos expression within the vasopressin neurons is a direct (NK3-R being activated are on the vasopressin neurons) or indirect (a synaptic) effect. Using internalization of the receptor as a marker of direct activation, we tracked the internalization of NK3-R on vasopressinergic neurons to first determine if senktide directly activates vasopressinergic neurons and second, whether NK3-R are internalized following injections of hypertonic saline, which is known to stimulate vasopressin release. Rats were fitted with cannulas in the anterior horn of the lateral ventricle and a catheter was inserted in the right femoral artery. Animals were given intraventricular injections of either 5  $\mu$ l of saline (n=8) or 400  $\mu$ g/5  $\mu$ l of senktide (n=12) and blood samples were taken. Rats were sacrificed 5, 20, or 120 minutes after the injection and brain tissue was processed for NK3-R and vasopressin immunoreactivity with a double immunofluorescent label. Animals treated with senktide had an increase in plasma vasopressin, whereas saline treated animals showed very little change. Following senktide injection, NK3-R immunoreactive dendrites of vasopressinergic neurons took on a bead-like appearance, which is indicative of receptor internalization, whereas, NK3-R remained membrane-bound in saline treated rats. NK3-R immunoreactive endosomes were also visible within the soma of senktide treated animals, whereas the saline treated animals showed much less soma internalization, which lead to the conclusion that an injection of senktide directly activates the vasopressin neurons within the PVN. To determine whether the NK3-R are activated following an injection of hypertonic saline experimental rats were given an intragastric load of 6 ml of 2M NaCl, while control rats were given 6 ml of 0.15M isotonic saline. All rats were sacrificed 45 minutes after the injection; brains were processed for both vasopressin and NK3-R immunoreactivity using a double immunofluorescent label, and visualized under a confocal microscope where the NK3-R internalizations on vasopressin neurons were quantified. After hypertonic saline injection, NK3-R immunoreactive dendrites on vasopressin neurons demonstrated a bead-like appearance, where as after the isotonic saline injection, NK3-R immunoreactive dendrites on vasopressin neurons were uniform in diameter and showed virtually no receptor internalization, leading to the conclusion that the NK3-R directly activates vasopressin neurons in the PVN in response to an injection of hypertonic saline. Supported by DK50586 and P20RR1564.

**EMBRYONIC EXPOSURE TO A VASOACTIVE INTESTINAL PEPTIDE (VIP) ANTAGONIST REDUCED SOCIAL APPROACH AND PREFERENCE FOR SOCIAL NOVELTY IN ADULT MICE.**

Joanna M. Hill<sup>1\*</sup>; Daniel Abebe<sup>2</sup>; Jacqueline N. Crawley<sup>1</sup>. <sup>1</sup>LBN, NIMH NIH, Bethesda, MD, <sup>2</sup>LDN, NICHD NIH, Bethesda, MD, USA

Autism is a developmental disorder of unknown etiology characterized by aberrant social behavior. A newly developed method of evaluating rodent social behavior reveals deficits in social approach behaviors and interest in social novelty. VIP regulates early postimplantation embryogenesis in the mouse and higher levels of VIP have been reported in blood of babies with autism. Blockage of VIP during embryogenesis results in microcephaly and developmental delays and, as adults, mice express atypical social responses in open field testing. In the current study, VIP was blocked during embryonic days 8-10 and testing occurred at 3 months. Following habituation of a test mouse to a 3-compartment chamber, an unfamiliar male mouse (S1) was introduced in a wire cage into a side chamber (sociability). After 15 min, a second unfamiliar male mouse (S2) was introduced in a wire cage into the other side chamber (social novelty). Time spent in exploration of the chambers and interaction with the unfamiliar mice was scored. Like controls, VIP antagonist treated mice spent more time in the chamber with S1 than in the empty chamber; however, they interacted with S1 significantly less than did control mice. When test mice chose between S1 and S2, control mice spent more time in the chamber with S2 and interacted with S2 half of the time in the chamber. VIP antagonist treated mice spent equal amounts of time in all three chambers and interacted with S1 and S2 equally and less than half of the time in each chamber. These data indicate that VIP antagonist treated mice participated in social interactions significantly less than control mice and did not differentiate between conspecifics, suggesting that this paradigm has potential as a model for social components of neurodevelopmental disorders such as autism. Supported by NICHD IRP and NIMH IRP.

**DIFFUSIBLE SIGNALS IN THE CIRCADIAN AND GnRH SYSTEMS.** Michael N. Lehman<sup>1</sup>, Eric L. Bittman<sup>2</sup>, and Heather J. Billings<sup>1</sup> <sup>1</sup>Dept. Cell Biology, Neurobiology & Anatomy and Neuroscience Program, University of Cincinnati College of Medicine, Cincinnati, Ohio; <sup>2</sup>Dept. Biology, University of Massachusetts, Amherst, Massachusetts

Volume transmission, via diffusible signals in the extracellular space, cerebrospinal fluid (CSF) or blood, has long been a controversial subject, but recent evidence suggests that diffusible signals may play a physiological role in communication for wide-acting systems that affect behavior and physiology. In this presentation, we will review recent findings concerning the role of diffusible signals, conveyed in CSF and peripheral blood, in the control of circadian rhythms and reproduction.

The suprachiasmatic nucleus (SCN) of the hypothalamus is the locus of a circadian clock that is essential for organizing daily rhythms in activity, autonomic, endocrine and cognitive functions. The SCN oscillator expresses a coherent phase that is communicated to oscillators located in other brain regions and the rest of the body. Transplantation studies have shown that the SCN communicates with other brain areas not only via neural efferents, but also through diffusible signals secreted into CSF or released locally within the brain parenchyma. In addition, recent studies suggest that communication between the SCN and oscillators in some peripheral tissues and endocrine organs are mediated by blood-borne signals while others are dependent on neural innervation. Although a number of candidates have emerged, the precise identity of circadian signals in CSF and blood still remains to be determined.

Gonadotropin-releasing hormone (GnRH) is a decapeptide that plays an essential role in the control of mammalian reproduction. GnRH is released into pituitary portal blood in a pulsatile manner to regulate the pituitary-gonadal axis, but is also secreted into the CSF in higher concentrations than would be expected by passive diffusion from brain sites or blood. GnRH axons penetrate the ependymal cell layer along the tubero-infundibular recess of the third ventricle, thus providing an anatomical basis for CSF release. In sheep, these axons arise from 30-60% of GnRH neurons, although it is not known yet whether these cells are the same as those that secrete GnRH into portal blood. One hypothesis is that GnRH secreted into CSF during the estrous cycle ensures synchronization of sexual behavior with ovulation. The targets for the CSF signal remain to be determined, as does whether blocking the site of GnRH release into CSF will interfere with sexual behavior. *Supported by the University of Cincinnati and NSF 0345752 to H.J.B.*

**DOES CEREBROSPINAL FLUID MELATONIN TRANSDUCE PHOTOPERIOD IN THE****BRAIN?** Benoît Malpoux, UMR INRA-CNRS-Université de Tours, Physiologie de la Reproduction et des Comportements, 37380 Nouzilly, France

Synthesised at night by the pineal gland, melatonin through the duration of its nocturnal secretion, is the primary transducer of photoperiodic information to the reproductive axis in seasonal breeders. It is present both in the blood and, in much higher concentrations (20 times), in the cerebrospinal fluid (CSF). This talk will address the origin and the potential role of CSF melatonin. Using several approaches, we tested the hypothesis that melatonin entered the CSF through the pineal recess, an evagination of the third ventricle. CSF melatonin concentrations are higher near the pineal gland than in the anterior part of the third ventricle, and decreased dramatically (80%) after sealing off the pineal recess. Moreover, ultrastructure and permeability analyses of the pineal-CSF interface showed that melatonin could reach the CSF either via delivery in situ by protruding pinealocytes contacting the CSF directly, or via extracellular secretion and interstitial fluid draining until the ventricular lumen. We demonstrated that CSF melatonin diffuses in the brain tissues and is the main source of this indoleamine in periventricular tissues, where melatonin receptors can be found. Concerning the respective role of blood and CSF melatonin in the photoperiodic integration, melatonin carried to the brain by the blood appears to be able to mediate the effects of photoperiod on reproduction but it appears that CSF melatonin may fine-tune this response both in terms of timing and amplitude.

**NEURAL ACTIONS OF THE INCRETIN PEPTIDE GLP-1.** TracyAnn Perry, Section of Drug Design & Development, Laboratory of Neurosciences, Gerontology Research Center, National Institute on Aging, Baltimore, MD.

Glucagon-like peptide-1 (7-36)-amide (GLP-1) is an endogenous 30-amino acid gut peptide, which binds at the GLP-1 receptor coupled to the cyclic AMP second messenger pathway. GLP-1 receptor stimulation enhances pancreatic islet  $\beta$ -cell proliferation, glucose-dependent insulin secretion, and lowers blood glucose and food intake in patients with type 2 diabetes mellitus. Not limited to the pancreas, the chemoarchitecture of GLP-1 receptor distribution in the brain of rodents and humans correlates with a central role for GLP-1 in the regulation of food intake. However emerging evidence suggests that stimulation of neuronal GLP-1 receptors plays an important role in regulating neuronal plasticity and cell survival. GLP-1 has been documented to induce neurite outgrowth and to protect against excitotoxic cell death and oxidative injury in cultured neuronal cells. Moreover, GLP-1 and exendin-4, a naturally occurring more stable analogue of GLP-1 that likewise binds at the GLP-1 receptor, were shown to reduce endogenous levels of amyloid- $\beta$  peptide ( $A\beta$ ) in mouse brain and to reduce levels of amyloid precursor protein (APP) in neurons. More recently we have demonstrated that these peptides offer protection against sensory neuropathy within the peripheral nervous system, suggesting a possible beneficial effect against the peripheral neuropathy associated with diabetes. Collectively these data suggest that treatment with GLP-1 or a related peptide beneficially affects a number of the targets associated with central and peripheral nervous system degeneration. Although much remains to be elucidated with regards the downstream signaling pathways involved in the pro-survival properties of GLP-1, we suggest a novel alternative and potentially valuable approach for the treatment of neurodegeneration.

**RESPONDING TO MIXED MESSAGES: INTERACTION OF PEPTIDERGIC, MECHANORECEPTIVE, AND NUTRIENT SIGNALS IN THE VAGAL AFFERENT CONTROL OF FOOD INTAKE.** R.C. Ritter., Dept. of VCAPP and Programs in Neuroscience, Washington State Univ., Pullman, WA 99164.

Maintenance of stable body weight depends on the degree to which controls of food intake accurately match energy intake with energy expenditure. Gastrointestinal peptides, and gut chemo- and mechano-sensitivity provide the brain with messages that forewarn of energy availability prior to nutrient absorption and assimilation. While hypothalamic peptides play important roles in control of food intake and energy balance, a growing number of observations indicate that some peptides exert control on food intake by modulating the activity of primary vagal afferent neurons. Integration and modulation of vagal sensory information, prior to its arrival in the forebrain could have profound implications for control of food intake. Recent results from our laboratories confirm that meal size is controlled by gut peptides, such as cholecystokinin (CCK), that are released prandially from the gastrointestinal tract. CCK, acting at CCK-1 receptors located on capsaicin-sensitive vagal afferents contributes to control of food intake by some intestinal nutrients, including some that do not stimulate CCK secretion. Furthermore, while CCK can reduce food intake directly by activating capsaicin-sensitive vagal afferents, it also can enhance or sensitize inhibition of feeding by gastric distension. In this regard, recent data from our lab indicates that CCK enhances responses to gastric distension by acting on previously unrecognized capsaicin-resistant vagal afferents. Thus, CCK seems to modulate or enhance responses to primary chemo- or mechano-receptive visceral afferents. Control of food intake by primary visceral afferents might also be influenced by messages typically associated with energy storage or adiposity. In fact we have found that leptin reduces meal size by acting directly on primary vagal afferent neurons that innervate the gastrointestinal tract. Furthermore, like the behavioral response to gastric distension, the vagally mediated reduction of food intake by leptin is markedly enhanced by CCK. Collectively, these observations indicate that primary vagal afferent neurons are important sites for integration of sensory and endocrine messages that controls food intake. An appreciation of the anatomical and physiological contexts and cellular mechanisms by which peptides, nutrients and mechanical stimuli influence vagal afferent signals will be essential for understanding their role in the control of food intake and could lead to new approaches for intervention disorders of food intake and energy balance.

**ACTIVATION OF THE HPA AND PLACENTAL AXIS INFLUENCES BIRTH OUTCOME AND THE HUMAN FETUS.** Curt A. Sandman<sup>1</sup>, Laura M Glynn<sup>1</sup>, Elysia P Davis<sup>1</sup>, Pathik Wadhwa<sup>1</sup>, Aleksandra Chicz-DeMet<sup>1</sup>, Christine Dunkel Schetter<sup>2</sup>, and Calvin Hobel<sup>3</sup>. <sup>1</sup>Department of Psychiatry, University of California, Irvine., <sup>2</sup>Department of Psychology, UCLA. <sup>3</sup>Cedar-Sinai, Los Angeles

Early experiences, including birth outcomes, exert life-long influences on health and well-being. These influences are termed programming and include risk for diabetes, heart disease, obesity and neurological diseases. There is growing evidence that peptides from the hypothalamic-pituitary-adrenal (HPA) axis influence separately birth outcome and early fetal development.

During the course of human pregnancy peptides synthesized by, and released from, the HPA axis gradually rise in the maternal plasma. We have found that at term corticotrophin releasing hormone (CRH), adrenocorticotropin (ACTH) and beta-endorphin (BE) are two- to ten-fold higher than non-pregnancy baselines. These peptides influence the timing of birth and are a conduit for the effects of environmental stress on the fetus. Maternal stress and the resulting physiological changes may result in decreased gestational duration and can directly influence the developing fetus. These effects may be independent but both can have long lasting (perhaps permanent) influences on development and are significant risks for mental retardation.

In two separate studies we found that fetal exposure to elevated CRH and to relatively high levels of the maternal opioid fragment (BE) was associated with significant impairment in fetal habituation to *ex utero* stimulation. In contrast, fetuses in maternal environments either with elevated ACTH or with balanced expression of POMC, exhibited the most rapid rate of habituation. We also recently found that elevated CRH during the second trimester is associated with human infant temperament at six weeks.

Elevated maternal CRF during the early third trimester apparently exerts (or reflects) at least two effects, one primarily influencing the length of gestation, and a second effect primarily influencing the fetal nervous system. Prenatal exposure to POMC fragments also influenced the course of habituation probably because of their direct effects on the well-developed fetal opiate receptor system. Our results implicate the maternal stress system early during human gestation as critical factors in fetal neurological development.

Supported by awards from the NIH (HD28413 and NS 41298)

**GUT FEELINGS: CELLULAR MECHANISMS FOR DETECTION AND INTEGRATION OF GASTROINTESTINAL AND METABOLIC SIGNALS BY PRIMARY VAGAL AFFERENTS.** S.M. Simasko. Program in Neuroscience, Washington State Univ., Pullman, WA 99164.

The entry of food into the gastrointestinal (GI) tract initiates a variety of mechanical, peptidergic, and nutritive signals that are important in the coordination of digestion and termination of a meal. While it is well established that intestinal release of cholecystokinin (CCK) and subsequent activation of vagal afferent fibers innervating the GI tract is of central importance to these processes, the detailed mechanism(s) by which CCK activate vagal afferent fibers, as well as how vagal afferent fibers respond to other GI generated signals, remains incompletely understood. To address possible cellular mechanisms underlying vagal activation we have used short term cultures of vagal afferent neurons isolated from nodose ganglia of rat. Patch-clamp measurements revealed that CCK depolarizes these neurons primarily as a result of activating a background depolarizing conductance. However, CCK secondarily causes a loss of a hyperpolarizing conductance. In contrast, leptin also activates these neurons, but leptin primarily causes the loss of a hyperpolarizing conductance, and secondarily causes activation of a depolarizing conductance. By the use of cytosolic calcium measurements we have found that subthreshold amounts of CCK and leptin can synergistically activate nodose neurons. These calcium measurements have also revealed that vagal afferent neurons respond directly to both long chain and short chain free fatty acids. These results demonstrate that vagal afferent fibers respond to a variety of peptidergic and metabolic signals and suggest that significant integration of these various pathways occurs at the level of vagal afferent activation. Further study of these mechanisms, and the adaptation of these mechanisms in various physiological states, should produce insights into control of food intake that may be useful in designing strategies to combat the problems associated with obesity.

**SEX, GnRH AND CEREBROSPINAL FLUID.** Donal C. Skinner<sup>1</sup>, Asher Albertson<sup>1</sup>, and Alain Caraty<sup>2</sup>.  
<sup>1</sup>Dept. Zoology & Physiology, University of Wyoming, Laramie, Wyoming; <sup>2</sup>INRA, Nouzilly, France

Gonadotropin-releasing hormone (GnRH), secreted by the hypothalamus, is the critical first component of the neuroendocrine reproductive axis that must be activated to ensure ovulation. This decapeptide is secreted into the hypophyseal portal circulation in pulses, the frequency of which is determined by the steroidal milieu, and as a large preovulatory surge. It has been shown in several species that GnRH is also released into the cerebrospinal fluid (CSF) and this release corresponds to that occurring in the hypophyseal portal blood. An overriding question is: does this CSF-GnRH have any physiological function?

We have found that centrally administered GnRH has no effect on its own secretion rejecting the hypothesis that CSF-GnRH modulates its own secretion. We have also shown that no CSF-GnRH enters the hypophyseal portal circulation and thus, has no direct effect on pituitary hormone secretion. However, we have compelling evidence that GnRH regulates sexual behavior but whether this is specifically through CSF has not been determined. Recently, we have determined the precise locations of GnRH receptor-expressing neurons within the brain using immunocytochemistry and high temperature antigen retrieval. Several areas (e.g. central grey, amygdala) that have been implicated previously in regulating sexual behavior contain intensely labeled neurons but other regions, such as the subfornical organ, also contain discrete populations of GnRH receptor-expressing cells. Which of these mediate the effects of GnRH on sexual behavior is not known.

### **The Regulation and Function of the Tachykinin *in vitro* and *in vivo* models of Epilepsy**

Spencer. E.M<sup>1</sup>, Howard.M<sup>1</sup>, Chandler.K<sup>2</sup>, Walker.A<sup>2</sup>, Kipar.A<sup>3</sup>, Thippeswamy.T<sup>3</sup>, and Quinn.J<sup>1</sup>, Physiology Department<sup>1</sup>, School of veterinary sciences<sup>3</sup>, University of Liverpool, Institute of Neurology, University college London<sup>2</sup>.

E-mail: ellys@liv.ac.uk, jquinn@liv.ac.uk

*In vivo* there are augmented levels of Substance P after status epilepticus. Modulation of neuropeptide expression results from changes in the transcription factor complement of the cell. The transcription factor Neuronal Restrictive Silencing Factor (NRSF/REST) is a key candidate modulating this plasticity of expression (Quinn *et al*, 2002). NRSF isoforms are induced in response to seizure in rodents (Palm *et al*, 1998) and we are correlating their expression with modulation of PPTA gene expression.

*In vitro* and *in vivo* seizure models have been used to analyse alterations in the expression of REST and PPTA. Methods include immunostaining, immunofluorescence, reporter gene constructs and Q-PCR using dissociated hippocampal neurons, organotypic slice models and *in vivo* kainic acid and perforant path seizure models. We will discuss our data correlating REST with PPTA expression.

In the future temporal and spatial topology of the neuropeptides and transcription factors will be compared in a variety of transgenic and virus vector models we have developed. We hope in this manner to delineate signal transduction pathways that result in the long-term differences to gene plasticity associated with the recurrence of seizure rather than the immediate mechanisms that result from the initial seizure.

**Acknowledgements:** This work was funded by The Wellcome Trust

#### **References:**

Palm, K., Belluardo, N., Metsis, M., & Timmusk, T. (1998). Neuronal expression of zinc finger transcription factor REST/NRSF/XBR gene. *J.Neurosci.* 18, 1280-1296.

Quinn, J.P., V.J. Bubb, Z.V. Marshall-Jones, and J.M. Coulson. 2002. Neuron restrictive silencer factor as a modulator of neuropeptide gene expression. *Regul Pept* 108: 135-41.

**DESIGNING AN ANTI-AMYLOIDOGENIC COMPOUND: A COMPREHENSIVE MOLECULAR MODELING AND *IN VITRO* INVESTIGATION OF DIANIONIC COMPOUNDS WITH THE A $\beta$  NEUROPEPTIDE.** Vanessa C. Stephenson<sup>1</sup> and Donald F. Weaver<sup>1,2,3</sup>; <sup>1</sup>Department of Chemistry, Dalhousie University, Halifax, NS, Canada; <sup>2</sup>Department of Medicine (Neurology), Dalhousie University, Halifax, NS, Canada; <sup>3</sup>School of Biomedical Engineering, Dalhousie University, Halifax, NS, Canada

The Alzheimer's-associated amyloid- $\beta$  (A $\beta$ ) peptide normally exists in an  $\alpha$ -helical form which is soluble and non-toxic to neurons. Upon conversion to  $\beta$ -sheet, however, A $\beta$  becomes insoluble, aggregating and subsequently precipitating from solution as fibrils and plaques in the self-propagating, self-amplifying cascade reaction known as amyloidosis.

It has been shown in previous binding studies that disulphonates have the ability to prevent the conversion of the  $\alpha$ -helical to the  $\beta$ -sheet form of A $\beta$ . This has provided the basis and motivation for further investigation in this area. Through the use of bioinformatics methods, including both molecular mechanical (Quanta® with CHARMM) and quantum mechanical (Gaussian98® using DFT/B3LYP/6-31+G2d) methods, interactions were modeled and binding studies have been performed involving the A $\beta$  peptide and various dianionic molecules. Molecules examined include various sulphonates, sulphinates, and carboxylates separated by 2-4 carbon atoms. Binding studies involving the peptide and each of the dianionics were evaluated at two different positions on the peptide—an interaction with His13 and His14 and an interaction with His 13 and Lys16. In all cases, preferential binding was found for the former interaction of two histidine residues (~17 kcal/mol stronger). Of the functional groups studied, the disulphinates appeared to have the strongest interactions, followed by dicarboxylates then disulphonates (-344.3, -290.0, and -252.2 kcal/mol, respectively). Higher-level, aqueous-phase, density-functional calculations also confirm this trend. Results were also evaluated in *in vitro* circular dichroism studies. This work provides insight into a rational design approach for the development of anti-amyloidogenic drugs.

## **Molecular dissection of the tachykinin signalling pathway by transgenesis: a Yeast Artificial Chromosome (YAC) approach**

S. Vasiliou, R. Morris\* and J. P. Quinn

Dept of Physiology and Human Anatomy & Cell Biology, \*Dept of Veterinary Science, University of Liverpool, UK

(e-mail: sylvia\_vasiliou@hotmail.com & jquinn@liv.ac.uk )

The tachykinin pathway is implicated in the regulation of diverse behavioural manifestations such as pain, anxiety, depression, stress responses and reward behaviours. Current preclinical rodent models are often compromised due to the differential expression of the tachykinins and/or their receptors between rodent and human and also inter-species differences in amino acid structures of the receptors that render them inappropriate targets for pharmaceutical intervention using drugs which are going into clinical trials

We have attempted to overcome some of these obstacles by inserting the human genes Pre-protachykinin-A and the tachykinin receptor NK1 into the mouse genome. Two separate Yeast Artificial Chromosomes (YACs) comprising the entire human PPT-A and NK1 loci were manipulated by the incorporation of the reporter genes lacZ and GFP, respectively, which would permit visualization of transgene expression.

The ability to manipulate YACs by homologous recombination and introduce human genes into animal models, will address questions regarding human gene regulation and function. Crossing the PPT-A and NK1 human alleles onto the relevant ablated genetic backgrounds generates an animal that only expresses the human genes. Such a "humanised" animal model will be a valuable tool for preclinical pharmacological and behavioural studies. Further with increased awareness of genetic polymorphisms predisposing to disease we will be able to address the function of human specific polymorphic domains in situ.

**VASOPRESSIN AND SUGAR-INDUCED BINGEING BEHAVIOR.** Cyrilla H. Wideman and Helen M. Murphy, Neuroscience Program, John Carroll University, Cleveland, OH 44118

In studies with human subjects, it has been hypothesized that abnormal entry of glucose into the central nervous system may exist in some chronic binge eaters of carbohydrates, as either a cause or consequence of binge eating. An experimental paradigm involving the development of sugar addiction in rats may serve as a useful animal model of carbohydrate binge eating in humans. One neuropeptide that may modulate excessive carbohydrate intake is vasopressin. Previous research in our laboratory demonstrated that vasopressin (VP) influences sugar intake and metabolism. The purpose of this study was to determine the role of VP in the development of sugar addiction, withdrawal, and bingeing. For four weeks, experimental VP-containing, Long-Evans (LE) and VP-deficient (DI) rats were provided with daily access to food for 12 hours. During weeks 2 and 4, animals had 12 hours access to 25% glucose in addition to food. Glucose and food intake were measured one and twelve hours following presentation. Animals with no access to glucose served as controls. During weeks 2 and 4, DI animals consumed more glucose than LE animals and there was an increase in glucose consumption in week 4 compared to week 2 for both stains. The increase of glucose from week 2 to week 4 was dramatic for DI rats. Both strains consumed 27-30% of their total glucose intake during the first hour of exposure in weeks 2 and 4, indicative of bingeing behavior. Whereas this percentage of intake for the first hour increased from week 2 to week 4 for DI animals, it decreased for LE animals. With respect to food intake, there were no differences between control animals and experimental animals during weeks 1 and 3; but in weeks 2 and 4, experimental animals consumed less food that control animals with no difference between the strains. Regarding body weight, all rats gained weight steadily throughout the experiment, but DI control animals weighed less than the other three groups. During week 3, rats previously exposed to glucose, were in a highly agitated state, with heads shaking and forepaws quivering with tremors. These behaviors are characteristic of withdrawal and were more evident in DI rats. In week 4, when glucose was restored, withdrawal symptoms dissipated. It is concluded that both strains displayed classic symptoms of addiction, withdrawal, and bingeing, but these symptoms were exacerbated in DI rats. Further research in this area may provide insights into important clinical phenomena.

**THE ROLE OF THE NEUROPEPTIDE GALANIN IN LEARNING AND MEMORY: FINDINGS FROM GALANIN TRANSGENIC AND GALR1 KNOCKOUT MICE.** Craig C. Wrenn, College of Pharmacy and Health Sciences, Drake University, Des Moines, IA 50311.

Galanin is a 29-amino acid neuropeptide that is widely distributed in the central and peripheral nervous systems. Galanin has attracted considerable interest in recent years in the study of learning and memory because of its overexpression in the nucleus basalis of Meynert in Alzheimer's disease (AD). This overexpression has led to the hypothesis that galanin contributes to the cognitive dysfunction of AD. Support for this hypothesis came from early experiments that used intracranial injection of galanin in rats. These studies showed that intraventricular, intrahippocampal, and intraseptal galanin produces deficits in a range of rodent behavioral paradigms of learning and memory. However, the relevance of these studies to the role of galanin in AD is limited by the reliance on exogenous galanin. We have recently tested the hypothesis that overexpression of endogenous galanin impairs cognitive function by testing learning and memory in transgenic mice (GAL-tg) that conditionally overexpress galanin in noradrenergic neurons. In GAL-tg mice, galanin peptide levels were approximately four times higher in the hippocampus and ten times higher in the cortex as compared to WT littermate controls. GAL-tg mice were impaired on several learning and memory tasks including the Morris water maze probe trial, the social transmission of food preference, and trace cued fear conditioning. These impairments were in the absence of any confounding genotype effects on general health, motor function, sensory ability, or attention. These data are consistent with the hypothesis that galanin overexpression results in cognitive dysfunction and suggests that GAL-tg mice are a useful model of AD.

The effects of galanin are mediated by three receptor subtypes which have been designated as GAL-R1, GAL-R2, and GAL-R3. The determination of which receptor subtype mediates the cognitive effects of galanin has remained elusive because of the lack of subtype-specific ligands. We have addressed the issue of galanin receptor function in learning and memory through the use of a line of mice that have a null mutation in the gene coding for GAL-R1 (GAL-R1 KO). GAL-R1 KO mice were normal on measures of general health, sensory and motor abilities, the Morris water task, social transmission of food preference, and standard delay fear conditioning. GALR1-KO mice were impaired on trace fear conditioning, as compared to WT littermate controls. This impairment was apparently sensitive to environmental factors as it was not replicated in GAL-R1 KO mice which were cannulated in the left lateral ventricle and extensively handled. Intraventricular injection (i.c.v.) of galanin (1nmole) had no effect on trace fear conditioning in cannulated GAL-R1 KO mice, while i.c.v. galanin significantly impaired trace fear memory. These data indicate that GAL-R1 is necessary for the deleterious effects of galanin on learning and memory.

**NEUROPEPTIDE S: A NOVEL MODULATOR OF AROUSAL AND ANXIETY.** Yanling Xu<sup>1</sup>, Rainer K. Reinscheid<sup>1</sup>, Salvador Huitron-Resendiz<sup>3</sup>, Steven J. Henriksen<sup>3</sup>, Luis de Lecea<sup>4</sup>, Olivier Civelli<sup>1,2</sup>.  
<sup>1</sup>Department of Pharmacology, <sup>2</sup>Department of Developmental and Cell Biology, University of California Irvine, Irvine, California 92697, USA; <sup>3</sup>Department of Neuropharmacology <sup>4</sup>Department of Molecular Biology, The Scripps Research Institute, La Jolla, California 92037.

Neuropeptide S (NPS) and its receptor, the NPS receptor (NPSR), is a newly orphanized G protein-coupled receptor (GPCR) system, for which little is known. We report here the pharmacological characteristics, anatomical distributions and physiological functions of this novel neuropeptide system. NPS binds its receptor with high affinity and induces transient increase in intracellular  $Ca^{2+}$  at low nanomolar concentrations. NPS precursor mRNA is found only in a few discrete nuclei, predominantly in a previously undefined cluster of cells located between the locus coeruleus (LC) and the Barrington's nucleus. The NPSR is widely expressed in many brain regions including the amygdala and the midline thalamic nuclei. Central administration of NPS increases locomotor activity. NPS also enhances wakefulness and suppresses all sleep stages including paradoxical (REM) sleep and slow wave sleep in rats, suggesting a profound arousal promoting effect. In addition, NPS reduces anxiety-like behaviors in mice exposed to four different stressful paradigms, demonstrating the anxiolytic-like effect of NPS. Our results indicate that the NPS system could be a new modulator of vigilance and anxiety. Furthermore, the unique anatomical pattern of NPS expression in the LC region indicates that the LC region encompasses distinct nuclei expressing different arousal-promoting neurotransmitters.

**Devin Adams**

University of Wyoming  
Dept. of Zoology & Physiology  
Dept. 3166, 1000 E. University Avenue  
Laramie, WY 82071  
Phone: 307-766-6446  
devina@uwyo.edu

**James Blevins**

VA Medical Center  
Research 151  
1660 South Columbian Way  
Seattle, WA 98108  
Phone: 206-277-6774  
Fax: 206-764-2164  
jblevin@uwashington.edu

**Doug Brenneman**

Johnson & Johnson Pharmaceutical  
Research & Development  
Welsh & McKean Roads  
Spring House, PA 19477  
Phone: 215-628-5774  
Fax: 215-540-4914  
Dbrennem@PRDUS.JNJ.COM

**Kimberly D'Anna**

University of Wisconsin  
1117 W. Johnson Street  
Madison, WI 53706  
Phone: 608-265-4155  
kldanna@wisc.edu

**Gaylen Edwards**

University of Georgia  
Physiology & Pharmacology  
College of Veterinary Medicine  
Athens, GA 30605  
Phone: 706-542-5854  
Fax: 706-542-0261  
gedwards@vet.uga.edu

**Asher Albertson**

University of Wyoming  
Department of Zoology & Physiology  
Dept. 3166, 1000 E. University Avenue  
Laramie, WY 82071  
Phone: 307-466-3331  
Fax: 307-766-5625  
asher693@uwyo.edu

**Michelle Block**

NICHD/NIH  
Room F 131  
111 Alexander Drive  
Research Triangle Park, NC 27709  
Phone: 919-541-5169  
Fax: 919-541-0841  
block@nichd.nih.gov

**Kristen Brunson**

HealthIQ, Inc.  
212 Murica Aisle  
Irvine, CA 92614  
Phone: 714-780-3851  
Fax: 714-621-0907  
kbrunson@uci.edu

**Robert Denver**

University of Michigan  
Department of Biology  
3077 Natural Science Building  
Ann Arbor, MI 48109-1048  
Phone: 734-936-6625  
Fax: 734-647-0884  
rdenver@umich.edu

**Bill Flynn**

University of Wyoming  
Dept. of Zoology & Physiology  
Box 3166, University Station  
Laramie, WY 82071  
Phone: 307-766-6446  
Fax: 307-766-2926  
flynn@uwyo.edu

**Nicole Gennet**

University of Liverpool  
Physiology  
Crown Street  
Liverpool, UK L69 6BX  
Phone: 01517945757  
gennet@liv.ac.uk

**Illana Gozes**

Tel Aviv University  
Sackler Faculty of Medicine  
Dept. of Clinical Biochemistry  
Tel Aviv, ISRAEL 69978  
Phone: 972-3-640-7240  
Fax: 972-3-640-8541  
igozes@post.tau.ac.il

**Maria Hadjimarkou**

NIH  
261 Congressional Lane, Apt. 516  
Rockville, MD 20852  
Phone: 301-402-3243  
Fax: 301-402-2200  
hadjimarkou@mail.nih.gov

**Joanna Hill**

NIH  
10/4D11  
9000 Rockville Pike  
Bethesda, MD 20892  
Phone: 301-496-4839  
Fax: 301-480-1164  
hilljoa@mail.nih.gov

**Michael Lehman**

University of Cincinnati  
College of Medicine  
3125 Eden Avenue  
Cincinnati, OH 45267-0521  
Phone: 513-558-7628  
Fax: 513-558-4343  
michael.lehman@uc.edu

**Warwick Giles**

University of Newcastle  
Discipline of Reproductive Medicine  
Faculty of Medicine & Health Sciences  
New South Wales, AUSTRALIA 2300  
Phone: 61 2 492 4385  
Fax: 61 2 492 4394  
giles@hunter.health.nsw.gov.au

**Liam Gray**

Wessex Neurological Centre  
Southampton General Hospital  
Southampton, UK SO16 6YD  
Phone: 44 23 8079 6057  
Fax: 44 23 8079 4148  
heather.johns@suht.swest.nhs.uk

**Gwendolen Haley**

University of Wyoming  
Dept. of Zoology & Physiology  
Dept. 3166, 1000 E. University Avenue  
Laramie, WY 82071  
Phone: 307-766-3129  
Fax: 307-766-5625  
ghaley@uwyo.edu

**Michael Kubek**

Indiana University  
School of Medicine  
635 Barnhill Drive  
Indianapolis, IN 46223

**Benoit Malpoux**

INRA UMR PRC  
Nouzilly, FRANCE 37380  
Phone: 33 247 42 79 44  
Fax: 33 247 42 77 43  
malpoux@tours.inra.fr

**Shawna McBride**

Univeristy of Wyoming  
Dept. of Zoology & Physiology  
Dept. 3166, 1000 E. University Avenue  
Laramie, WY 82071  
Phone: 307-766-3129  
Fax: 307-766-5625  
smcbride@uwyo.edu

**Helen Murphy**

John Carroll University  
Department of Psychology  
20700 North Park Boulevard  
Cleveland, OH 44118  
Phone: 216-397-4359  
Fax: 216-397-1633  
hmurphy@jcu.edu

**TracyAnn Perry**

Amylin Pharmaceuticals  
9360 Towne Centre Drive  
San Diego, CA 92121  
Phone: 858-309-7475  
Fax: 858-824-7726  
tracyann.perry@amylin.com

**John Quinn**

University of Liverpool  
Medical School, Chair of Neurobiology  
Physiology & Human Anatomy Cell Biology  
Liverpool, UK L69 3BX  
Phone: 44 151 794 5498  
Fax: 44 151 794 5517  
jqinn@liv.ac.uk

**Andy Russo**

University of Iowa  
Dept. of Physiology & Biophysics  
5-432 BSB, 51 Newton Road  
Iowa City, IA 52242  
Phone: 319-335-7872  
Fax: 319-335-7330  
andrew-russo@uiowa.edu

**James Miller****Theo Peeters**

Katholieke University  
Department of Medical Research  
Gut Peptide Laboratory  
Gasthuisberg Leuven, BELGIUM B-3000  
Phone: 32 16 345757  
Fax: 32 16 345939  
theo.peeters@med.kuleuven.ac.be

**James Peters**

Washington State University  
Department of VCAPP  
Pullman, WA 99164-6520  
Fax: 509-335-4650  
peterj@vetmed.wau.edu

**Robert Ritter**

Washington State University  
Department of VCAPP  
College of Veterinary Medicine  
Pullman, WA 99164-6520  
Phone: 509-335-8114  
Fax: 509-335-4659  
britter@vetmed.wsu.edu

**Curt Sandman**

University of Cal., Irvine  
Dept. of Psychiatry  
2501 Harbor Boulevard, 5A  
Costa Mesa, CA 92626  
Phone: 714-957-5435  
Fax: 714-957-5354  
casandma@uci.edu

**Tom Sellers**

Lemuel Shatteck  
170 Morton Street  
Jamaica Plain, MA 02130

**Harlan Shannon****Steve Simasko****Donal Skinner**

University of Wyoming  
Department of Zoology & Physiology  
Laramie, WY 82071  
Phone: 307-766-4922  
Fax: 307-766-5625  
dcs@uwyo.edu

**Michael Smith**

Penn State College of Medicine  
Neural & Behavioral Sciences, H-181  
Hershey, PA 17033  
Phone: 717-531-3758  
Fax: 717-531-6916  
mes32@psu.edu

**Eleanor Spencer**

University of Liverpool  
Physiology  
Crown Street  
Liverpool, UK L69 3BX  
Phone: 44 151 794 5757  
ellys@liv.ac.uk

**Vanessa Stephenson**

Dalhousie University  
Department of Chemistry  
6295 Coburg Road  
Halifax, Nova Scotia, CANADA B3H 4J3  
Phone: 902-494-7021  
Fax: 902-494-1310  
vestephen@dal.ca

**James Stewart**

University of Liverpool  
Department of Medical Microbiology  
Duncan Building, Daulby Street  
Liverpool, UK L69 3GA  
Phone: 44 151 794 7596  
Fax: 44 151 706 5805  
j.p.stewart@liv.ac.uk

**Fleur Strand**

Carroll & Milton Petrie  
Professor of Biology, Emerita  
340 East 64th Street  
New York, NY 10021-7511  
Phone: 212-832-9774  
Fax: 212-319-6233  
fleur.strand@nyu.edu

**Thimmasettappa Thippeswamy**

University of Liverpool  
Dept. of Veterinary Preclinical Sciences  
Brownlowhill and Crown Street  
Liverpool, UK L69 7ZJ  
Phone: 44 151 794 4242  
Fax: 44 151 794 4243  
tswamy@liv.ac.uk

**Claude Wasterlain**

VA Medical Center (127)  
11301 Wilshire  
West Los Angeles, CA 90073  
Phone: 310-268-3595  
Fax: 310-268-4611  
wasterla@ucla.edu

**Cyrilla Wideman**

John Carroll University  
Department of Biology  
20700 North Park Boulevard  
Cleveland, OH 44118  
Phone: 216-397-4250  
Fax: 216-397-1633  
cwideman@jcu.edu

**Mark Witten**

University of Arizona  
Department of Pediatrics, College of Med  
1501 N. Campbell Avenue  
Tucson, AZ 85724  
Phone: 520-626-2610  
Fax: 520-626-4993  
mwitten@peds.arizona.edu

**Simon Wong**

University of Arizona  
Dept. of Pediatrics, School of Med.  
1501 N. Campbell Avenue  
Tucson, AZ 85724  
Phone: 520-626-6572  
Fax: 520-626-4993  
shengjun@emial.arizona.edu

**Craig Wren**

Drake University  
2507 University Avenue  
Des Moines, IA 50311  
Phone: 515-271-3326  
Fax: 515-271-1867  
craige.wrenn@drake.edu

**Yanling Xu**

University of California, Irvine  
6289 Adobe Circle Road, South  
Irvine, CA 92617  
Phone: 949-824-2591  
Fax: 949-824-4855  
yanlingx@uci.edu