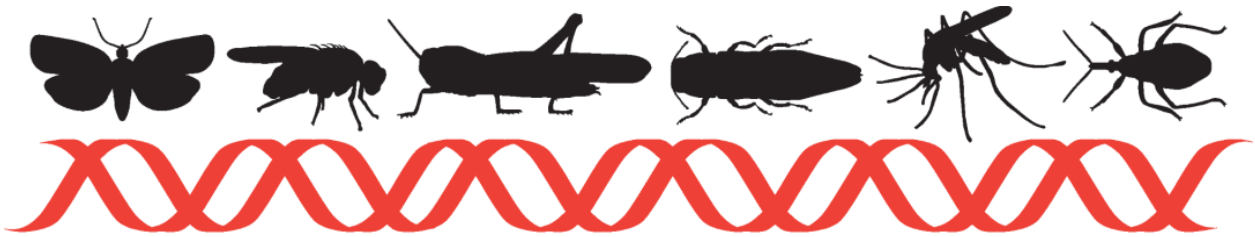

INSECT BIOTECH CONFERENCE

Insect Biotech Conference 2021

June 2-3, 2021

Schedule and Contributed Abstracts

Insect Biotech Conference 2021

Sponsors:

Insect Biotech Conference – June 2, 2021

Day 1 Conference Schedule

(NOTE: all times shown in EST)

12:00 – 12:55 pm **Breakfast/lunch social and networking session**

1:00 pm **Welcome and Opening Remarks**

1:10 pm **PLENARY SPEAKER: PROF. KARLA KAUN**

To drink or Not(ch) to drink: How alcohol molecularly influences memory circuits [Page 8]

Kristin M. Scaplen[1], Mustafa Talay[1,2], Emily Petruccelli[1,3], Nicolas Ledru[1,4], Tariq Brown[1] Gilad Barnea[1], Karla R. Kaun[1]

[1]Department of Neuroscience, Brown University, Providence, RI.
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Session #1 (Long talks) (Chair: Dennis Kolosov)

2:00 pm **Exploring the role of glycoprotein hormone GPA2/GPB5 on the diuretic process in the medically important insect, *Rhodnius prolixus*. [Page 9]**
A. Al-Dailami, J. Leyria, I. Orchard, AB. Lange
Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada.

2:20 pm **Comparing the brain transcriptome of worker and queen honey bees (*Apis mellifera*) during acts of aggression. [Page 10]**
K. Galang and A. Zayed
Department of Biology, York University, Toronto, Ontario, Canada

2:40 pm **The hydrostatic organ of the *Chaoborus* midge larva is a pH driven mechanochemical engine regulated by cAMP. [Page 11]**
E.K.G. McKenzie[1], G. Kwan[2], M. Tresguerres[2], P.G.D. Matthews[1]
[1]Department of Zoology, University of British Columbia, Vancouver British Columbia, Canada
[2]Scripps Institution of Oceanography, University of California San Diego, San Diego, USA

3:00 pm **Investigating the potential for urbanization to alter mosquito seasonal physiology. [Page 12]**
L. Fyie, M. Gardiner and M. Meuti
Department of Entomology, Ohio State University, Columbus, Ohio, United States of America

3:20-3:40 pm **BREAK**

Session #2 (Short talks) (Chair: Leena Thorat)

3:40 pm **Comprehensive transcriptomic survey of animal epithelia reveals the presence of voltage-gated, ligand-gated, mechanosensitive, and gap junction channels. [Page 13]**
T Karpinski, A Castaneda, D Kolosov
Department of Biological Sciences, California State University San Marcos, San Marcos, California, USA.

3:50 pm **No consequences of microplastic ingestion on development of the decorated cricket (*Gryllodes sigillatus*). [Page 14]**
S. Fudlosid, H. MacMillan, and M. Muzzatti
Department of Biology, Carleton University, Ottawa, Ontario, Canada

4:00 pm **Measuring the Effect of Royal Jelly on the Seasonal Responses of *Culex pipiens*. [Page 15]**
O. Bianco[1], M. Klein[2] and M. Meuti[1]
[1]Department of Entomology, The Ohio State University, Columbus, Ohio, United States
[2]Department of Food Science and Technology, The Ohio State University, Columbus, Ohio, United States

4:10 pm **Anoxic spreading depolarization in locust CNS: the role of anaerobic glycolysis. [Page 16]**
Y. Wang and R. M. Robertson
Department of Biology, Queen's University, Kingston, ON, Canada

4:20 pm **Identification and characterization of the Halloween and ecdysone-responsive genes in the ovaries of *Rhodnius prolixus*. [Page 17]**
S. Benrabaa, I. Orchard and A. Lange
Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada

4:30 pm **Effects of Helicokinin-3 on Malpighian tubule function in the larval *Trichoplusia ni*. [18]**
Serna E, Gonzalez I, Solorio M, Kolosov D
Department of Biological Sciences, California State University San Marcos, San Marcos, California, USA.

4:40 pm **Voltage-gated calcium channels in epithelia of mosquitoes – effects of rearing environmental salinity in larvae and blood-feeding in adults. [19]**
S Farrell, N Ramirez, M Lopez-Sanchez, D Kolosov
Department of Biological Sciences, California State University San Marcos, San Marcos, California, USA.

4:50 pm **The effects of fusion on the larvae of *Chironomus riparius*: The search for alternatives to road salt. [Page 20]**
S J Silver, J P Paluzzi, A Donini
Department of Biology, York University, Toronto, ON Canada.

Insect Biotech Conference – June 3, 2021

Day 2 Conference Schedule

(NOTE: all times shown in EST)

12:00 – 12:55 pm **Breakfast/lunch social and networking session**

Session #3 (Long talks) (Chair: Andrew Donini)

1:00 pm **Distribution of the adipokinetic hormone/corazonin-related peptide in the disease vector, *Aedes aegypti* and identification of key receptor residues necessary for ligand activation. [Page 21]**
S. Afifi and J.P. Paluzzi
Department of Biology, York University, Toronto, ON, Canada.

1:20 pm **The Influence of a Blood or Nectar Meal on Aquaporin Abundance in the Disease Vector Mosquito, *Aedes aegypti*. [Page 22]**
B.N. Picinic, A. Donini, and J.P. Paluzzi
Department of Biology, York University, Toronto, Ontario, Canada

1:40 pm **Elucidating the role of the V-type H+ ATPase in CAPA-mediated inhibition in *Aedes aegypti* Malpighian tubules. [Page 23]**
F. Sajadi and J.P. Paluzzi
Department of Biology, York University, Toronto, Ontario, Canada.

2:00 pm **Expression profiling and characterization of CCHamide2 neuropeptide and its G-protein coupled receptor in the yellow fever mosquito, *Aedes aegypti*. [Page 24]**
J. Tan and J.P. Paluzzi
Department of Biology, York University, Toronto, Ontario, Canada.

2:20 pm **Insights into the physiological significance and bioproperties of underwater silks spun by chironomid midges. [Page 25]**
L. Thorat[1], E. Joseph[2,3], A. Nisal[2,3], E. Shukla[4], A. Ravikumar[5], B. B. Nath[6]
[1]Department of Biology, York University, Canada
[2]Polymer Science and Engineering Division, CSIR-NCL, India
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[4]National Centre for Cell Science, India
[5]Institute of Bioinformatics and Biotechnology, Savitribai Phule Pune University, India
[6]Stress Biology Research Laboratory, Department of Zoology, Savitribai Phule Pune University, India

2:40-3:00 pm **BREAK**

Session #4 (Long talks) (Chair: Jean-Paul Paluzzi)

3:00 pm **Identifying and characterizing an IMD candidate in *Rhodnius prolixus* using 3D modeling, phylogenetic conservation, and gene knockdown approaches. [Page 26]**
N. Salcedo-Porras[1], D. Giron-Ceron[1], K. Elliot[1], C. Umana-Diaz, P. L. Oliveira[2] and C. Lowenberger[1]
[1]Centre for Cell Biology, Development and Disease, Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6, Canada, keenan_elliot@sfu.ca (K.E.), diana_giron_ceron@sfu.ca (D.G.C), clowenbe@sfu.ca (C.L.)
[2]Instituto de Bioquímica Médica, Universidade Federal do Rio de. Janeiro, CCS, Ilha do Fundão, Rio de Janeiro, Brazil

3:20 pm **Voltage-gated ion channels in animal epithelia – what gives? [Page 27]**
D. Kolosov
Department of Biological Sciences, California State University San Marcos, San Marcos, California, USA.

3:40 pm **The effects of nitric oxide on the cardiovascular system and immune response of the mealworm. [Page 28]**
M. Nissan and R. da Silva
Department of Biology, McMaster University, Hamilton, Ontario, Canada

4:00 pm **Discovery of the elusive *Drosophila* ion transport peptide (ITP) receptor. [Page 29]**
M. Zandawala[1], F. Sajadi[4], A. Matei[4], F. McEwan[3], S. Kondo[2], D. Nassel[3], J.P. Paluzzi[4]
[1]Dept of Neuroscience, Brown University, Providence, USA
[2]Invertebrate Genetics Laboratory, National Institute of Genetics, Mishima, Japan
[3]Dept of Zoology, Stockholm University, Stockholm, Sweden
[4]Dept of Biology, York University, Toronto, Canada

4:20-4:30pm **BREAK**

Session #5 (Short talks) (Chair: Farwa Sajadi)

4:30 pm **Characterization of RYamide Neuropeptide and Its Receptor in the Human Disease Vector, *Aedes aegypti*. [Page 30]**
T. Nguyen and J.P. Paluzzi
Department of Biology, York University, Toronto, Ontario, Canada.

4:40 pm **Identification of a tachykinin receptor and its implication in metabolism in *Rhodnius prolixus*, a Chagas disease vector. [Page 31]**
Haddad, A, Leyria, J. and Lange, AB
Department of Biology, University of Toronto Mississauga, Ontario, Canada

4:50 pm **Identifying the glycosylation site on *Drosophila melanogaster* ion transport peptide and its potential physiological role. [Page 32]**
M. Agard, M. Kooner, A. Matei and J.P. Paluzzi
Department of Biology, York University, Toronto, ON,

5:00 pm End of IBC 2021 virtual conference

Abstracts:

To drink or Not(ch) to drink: How alcohol molecularly influences memory circuits

Kristin M. Scaplen[1], Mustafa Talay[1,2], Emily Petruccelli[1,3], Nicolas Ledru[1,4], Tariq Brown[1] Gilad Barnea[1], Karla R. Kaun[1]

[1]Department of Neuroscience, Brown University, Providence, RI.
[2]Current address: Department of Molecular and Cell Biology, Harvard University, Cambridge, MA.
[3]Current address: Department of Biology, Southern Illinois University, Edwardsville, IL.
[4]Current address: MSTP Program, Washington University, St Louis, MO.

Investigating how drugs of abuse affect molecular mechanisms within reward memory circuits is key to understanding how cravings are acquired and expressed. Combining forward genetics, transcriptomic and detailed circuit mapping approaches in *Drosophila*, we've shown how alcohol influences the a highly conserved cell-signaling pathway, called Notch, to affect gene expression required for memory formation. Our work provides direct evidence that alcohol induces immediate changes in Notch signaling. This leads to gene expression changes required for neuronal plasticity in memory-encoding neurons. Activation of Notch signaling also correlates with expression of alternative transcript isoforms of key genes that regulate multiple forms of memory. This process was very dynamic, and appeared to result in different transcript isoforms of the same gene being expressed after formation of alcohol memory. This suggests that alcohol alters gene expression while memories are becoming encoded, potentially strengthening memories for alcohol. Using circuit mapping techniques, we are investigating how this molecular change can influence a dynamic shift from circuits that form memories to circuits that initiate cue-induced behavioral responses.

Exploring the role of glycoprotein hormone GPA2/GPB5 on the diuretic process in the medically important insect, *Rhodnius prolixus*.

A. Al-Dailami, J. Leyria, I. Orchard, AB. Lange

Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada.

Glycoprotein hormones are formed by the heterodimerization of alpha and beta subunits. They mediate a wide range of physiological functions such as metabolism, reproduction, and development. In vertebrates, glycoprotein hormones include follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH), among others. The last glycoprotein hormone discovered was thyrostimulin, which is formed by the dimerization of GPA2 with GPB5 subunits. To date, the functional role of thyrostimulin (GPA2/GPB5) in vertebrates has not been fully elucidated. However, recent reports in invertebrates, specifically in holometabolous insects, suggest that GPA2/GPB5 plays a critical role in development and diuresis. In this study, we characterize the glycoprotein hormone (GPA2/GPB5) and its receptor (LGR1) in *Rhodnius prolixus*, which is a hemimetabolous insect and the vector of Chagas disease. We also investigate the physiological roles for this glycoprotein hormone-signalling pathway in fifth instar R. prolixus, specifically in relation to feeding and diuretic processes, both of which have high epidemiological relevance. Both subunit transcripts, GPA2 and GPB5, and LGR1 transcripts are present in a variety of tissues, with greatest expression of the subunits in the central nervous system (CNS), whereas LGR1 expression is highest in the Malpighian tubules (MT). Results from temporal qPCR analyses are consistent for the subunits and the receptor, showing a decrease in transcript expression 24 h after feeding in the CNS and MTs, respectively, followed by an increase in transcripts as the days after feeding advance. In order to assess the role of this glycoprotein hormone in diuresis and feeding, we silenced the expression of the LGR1 transcript using RNA interference, and at 7 days post-injection, insects with reduced LGR1 expression showed greater weight loss and mortality rate in both fed and unfed nutritional states. In addition, insects with reduced LGR1 consumed a significantly smaller blood meal and the mortality rate was greater. The results suggest that GPA2/GPB5 may play a role controlling R. prolixus feeding and diuresis.

This work was supported by NSERC Discovery Grants to ABL and IO.

Comparing the brain transcriptome of worker and queen honey bees (*Apis mellifera*) during acts of aggression.

K. Galang and A. Zayed

Department of Biology, York University, Toronto, Ontario, Canada"

The aggressive behaviour of the Western honey bee, *Apis mellifera*, is prototypically characterized by stinging. A worker bee will sting intruders to defend her hive. This behaviour is frequently categorized as altruistic as she sacrifices herself to protect her sisters and queen. In sharp contrast, a virgin queen bee stings to directly obtain something for herself, reproductive control. A newly emerged virgin queen will seek out and destroy her sister queens to ensure she is the sole reproductive member. For this reason, queen stinging can be labelled as selfish aggression.
The genetic and molecular bases of altruistic worker aggression in honey bees have been extensively studied but, to my knowledge, there have not yet been any studies done regarding the genetic or molecular bases of selfish queen aggression. Elucidating this knowledge gap is helpful in understanding how these two contrasting types of aggression, altruistic vs selfish, influence the neuro-transcriptomic state of bees and will allow better understanding of the molecular and evolutionary bases of altruistic aggression. The altruism displayed in the honey bee is a unique trait and it is not currently clear how this behaviour evolved. I test the hypothesis that altruistic aggression is transcriptomically different from that of selfish aggression by comparing the brain gene expression patterns of worker honey bees against that of queen honey bees, both before and after an intruder challenge. This comparison should provide a basis for a more in-depth understanding of how situational context (altruistic vs selfish) affects aggression.

This research was supported by NSERC and OGS.

The hydrostatic organ of the *Chaoborus* midge larva is a pH driven mechanochemical engine regulated by cAMP.

E.K.G. McKenzie[1], G. Kwan[2], M. Tresguerres[2], P.G.D. Matthews[1]

[1]Department of Zoology, University of British Columbia, Vancouver British Columbia, Canada
[2]Scripps Institution of Oceanography, University of California San Diego, San Diego, USA

The aquatic larvae of the *Chaoborus* midge are the only insects which achieve neutral buoyancy. They remain suspended in the water column of lakes and ponds by regulating the volume of four internal air-filled bladders (air-sacs) that are derived from the tracheal system. Here we describe the physiological and mechanical aspects of this unique buoyancy regulation system. Instead of secreting gas into the air-sacs, the air-sac wall itself expands and contracts in response to changes in its pH, altering the volume of the lumen. This pH-sensitivity is due to the presence of bands of resilin, a pH sensitive protein, in the air-sac wall. Bands of resilin alternate with bands of cuticle, and this architecture of alternating contractile and non-contractile material restricts changes in volume to only one dimension. This causes the air-sac to behave as a pH-muscle, converting chemical energy in the form of changes in pH directly into mechanical work. The pH of the air-sacs is regulated by an enveloping endothelium containing vacuolar type H+ APTase (VHA) as shown by immunofluorescence. Bafilomycin, a VHA inhibitor, prevents proton pumping and causes air-sac expansion. In vitro application of either 8-Bromo-cAMP or forskolin + IBMX also results in expansion of the air-sac, and therefore we hypothesize that cAMP activates exchange proteins and/or ion channels which dissipate the VHA-generated pH gradient across the endothelium. The cAMP analogue 8-pCPT-2'-O-Me-Cyclic AMP, which specifically targets exchange proteins directly activated by cAMP (Epac) drives air-sac expansion more effectively than activators of PKA, indicating a role for Epac in air-sac alkalinization. This system is the first known example of a pH-driven mechanochemical engine in nature.

This research was supported by NSERC and a B.C. Graduate Scholarship

Investigating the potential for urbanization to alter mosquito seasonal physiology.

L. Fyie, M. Gardiner and M. Meuti

Department of Entomology, Ohio State University, Columbus, Ohio, United States of America

Cities experience unique environmental conditions, such as light pollution caused by artificial light at night (ALAN) and increased temperatures caused by the Urban Heat Island (UHI), that could interfere with environmental cues for seasonal responses in temperate insects. Both ALAN and the UHI effect advance spring phenology in a number of taxa. However, investigations of how they may influence late-season seasonal responses remain limited. The Northern house mosquito, *Culex pipiens*, is a primary vector of West Nile virus (WNV). Females of this species enter a developmental arrest, or diapause, in response to short days and low temperatures during autumn. Diapausing females cease reproductive development and blood-feeding, and instead accumulate fat, halting disease transmission. Both ALAN and the UHI have the potential to interfere with the perception of changing seasons, inhibit diapause initiation, and thus extend the active mosquito biting season. In two separate lab experiments, we reared mosquitoes in at autumn daylengths in environmental chambers and found that exposure to both dim ALAN (~4 lux) and temperatures that simulated the UHI in temperate biomes during the winter alters seasonal phenotypes. Both ALAN and the UHI increased ovarian development and blood-feeding. However, only exposure to ALAN decreased fat accumulation whereas the UHI had no impact. Taken in tandem, these results indicate that urbanization has the potential to interfere with normal seasonal responses in *Cx. pipiens*. This contributes to growing knowledge on how cities affect public health and provide important information for predicting disease risk and managing mosquitoes for city residents.

Comprehensive transcriptomic survey of animal epithelia reveals the presence of voltage-gated, ligand-gated, mechanosensitive, and gap junction channels.

T Karpinski, A Castaneda, D Kolosov

Department of Biological Sciences, California State University San Marcos, San Marcos, California, USA.

Epithelia are multifunctional tissues of animals that are often faced with rapid changes in external or systemic ion content. Recent work employing RNAseq approaches determined that voltage-gated, ligand-gated, mechanosensitive, and gap junction are expressed in epithelia of many animals, indicating that these molecular components likely constitute a universal epithelial toolkit for rapid autonomous regulation of epithelial function. Transcriptomic approach allows to not only detect the expression of these channels in animal epithelia, but to determine what affects their transcript abundance levels. Previous studies have determined that these channels are expressed in epithelia of mammals, teleosts, insects, mollusks and echinoderms. The objective of our study was to determine whether clades not investigated previously also express voltage-gated, ligand-gated, mechanosensitive, and gap junction channels in their epithelia and identify what factors alter their transcript abundance.

Acknowledgements: This research was supported by startup funds from CSUSM and internal research grant monies.

No consequences of microplastic ingestion on development of the decorated cricket (*Gryllodes sigillatus*).

S. Fudlosid, H. MacMillan, and M. Muzzatti

Department of Biology, Carleton University, Ottawa, Ontario, Canada

Microplastics (MP) are a growing concern as environmental contaminants as they are now considered ubiquitous in our ecosystems. Microplastics have been confirmed to be present in terrestrial environments, yet the majority of studies have focused on the adverse effects of MPs on aquatic biota. We tested the effect of prolonged dietary microplastic exposure on the growth and survival of the decorated cricket *Gryllodes sigillatus*. Freshly hatched crickets were fed fluorescent polyethylene MP beads (75-105μm diameter) mixed into the cricket feed at concentrations of 0, 2.5, 5, and 10% w/w throughout development until adulthood. Weight and body length were measured weekly and MP ingestion was confirmed through fluorescence microscopy. Surprisingly, we found no effect of MP ingestion on growth rate or final body size. These results suggest that high concentrations of MP beads can be passed through the cricket's gut without a substantial negative effect on their growth and development time. Although prolonged MP ingestion did not affect the development of the crickets, questions remain on its effects on reproduction and bioaccumulation.

Acknowledgements: Thank you for all the support and encouragement from my peers in the MacMillan lab. This research was supported by NSERC.

Measuring the Effect of Royal Jelly on the Seasonal Responses of *Culex pipiens*.

O. Bianco[1] , M. Klein[2] and M. Meuti[1]

[1]Department of Entomology, The Ohio State University, Columbus, Ohio, United States
[2]Department of Food Science and Technology, The Ohio State University, Columbus, Ohio, United States

Females of the Northern house mosquito, *Culex pipiens*, enter diapause in response to a decrease in absolute day length and environmental temperature. Diapausing female mosquitoes adjust their feeding patterns, exhibiting a preference for sugar-rich sources like nectar rather than human or animal blood, thus reducing the transmission of diseases. During diapause, females of *Cx. pipiens* upregulate a protein referred to as Major Royal Jelly Protein 1 (MRJP1). This protein is highly abundant in royal jelly, a substance produced by honey bees, Apis mellifera, that is fed to future queens throughout larval development and stimulates their longevity and reproduction. We investigated how supplementing the diets of both diapausing and nondiapausing females of Cx. pipiens with royal jelly affects their diapause status, protein content, longevity, and metabolic profile. We reared mosquitoes under both short-day, diapause-inducing, and long-day, diapause-averting conditions and allowed females to feed on either sugar water or royal jelly. Furthermore, we knocked down MRJP1 using RNA Interference (RNAi) and determined the effect on diapause status and longevity. Once females were one week old, we measured their primary egg follicle lengths and fat content to assess their diapause status. We found that feeding royal jelly to females significantly reduced the egg follicle lengths of non-diapausing females, suggesting that these females entered a diapause-like state. Additionally, RNAi directed against MRJP1 significantly increased egg follicle length of diapausing females, suggesting that these females averted diapause. I hypothesize that supplementing the diet of female mosquitoes with royal jelly will lead to an increase in lifespan, while knocking down MRJP1 with RNAi will lead to a decrease in lifespan. These findings could lead to novel developments to prevent mosquitoes from biting humans and animals and thereby reduce disease transmission.

This work was supported by state and federal funds appropriated to The Ohio State University, College of Food, Agricultural, and Environmental Sciences, Ohio Agricultural Research and Development Center.

Anoxic spreading depolarization in locust CNS: the role of anaerobic glycolysis.

Y. Wang and R. M. Robertson

Department of Biology, Queen's University, Kingston, ON, Canada

The migratory locust (*Locusta migratoria*) can survive through periods of environmental anoxia by entering a reversible state of coma, marked by neuromuscular inactivity and metabolic depression. Spreading depolarization (SD) is the mechanism underlying coma induction in the locust central nervous system (CNS), which involves the near complete depolarization of neural cells and the silencing of electrical activity. During severe metabolic insults such as anoxia, glycolytic flux is considered to be neuroprotective; yet the precise involvement of anaerobic glycolysis in anoxic SD progression and recovery remains elusive. Here, we investigate the role of anaerobic glycolysis in locust anoxic SD through pharmacological manipulations of glycolytic capacity. Semi-intact locusts were pre-treated with either glucose, trehalose, or monosodium iodoacetate (MIA, GAPDH inhibitor) before N2-induced anoxia. The characteristics of SD onset and recovery were studied with an electrophysiological approach. We found that glycolytic capacity minimally influences the time course of SD induction; however, glucose considerably enhanced SD recovery rate and reduced the amplitude of the negative DC shift. Moreover, MIA slowed down the recovery and depressed the extracellular DC potential after anoxia. Taken together, these observations suggest anaerobic glycolysis may serve to maintain ion homeostasis during coma and provide a significant energy boost during SD recovery. Furthermore, MIA potentially exacerbates anoxia-dependent oxidative damage to the perineurial glia, resulting in depression of DC potential. Our research contributes to the broader mechanistic understanding of insect anoxic SD, and provides a basis for further exploration in the interplay between neural anaerobic metabolism and SD parameters – an often overlooked aspect in both insect and mammalian SD literatures.

Acknowledgements: This research was funded by NSERC

Identification and characterization of the Halloween and ecdysone-responsive genes in the ovaries of *Rhodnius prolixus*.

S. Benrabaa, I. Orchard and A. Lange

Department of Biology, University of Toronto Mississauga, Mississauga, Ontario, Canada

Rhodnius prolixus is a blood-gorging insect, which is medically important since it causes Chagas disease by transmitting the *Trypanosomes cruzi* parasite via its feces and urine after a blood meal. In adult females, the blood meal and ecdysteroid hormone (20-hydroxyecdysone) are involved in the growth of the ovary and development of eggs. Halloween genes are necessary for ecdysteroid synthesis since they code for cytochrome P450 enzymes in the ecdysteroidogenic pathway. The ecdysteroid receptor (EcR/USP) binds 20-hydroxyecdysone, resulting in serial activation of ecdysone-responsive genes. We identified and characterized the Halloween genes, Neverland, CYP18a1, and eight ecdysone-responsive genes in the *R. prolixus* ovary using transcriptomic data. We used BLAST to compare transcriptome sequences with other arthropod sequences to identify similar transcripts. Our results indicate the Halloween genes and ecdysone-responsive genes are present in the ovary of *R. prolixus*. Future work will quantify Halloween gene expression and ecdysone-responsive gene expression in the ovary following a blood meal.

Acknowledgments: Supported by NSERC Discovery Grants to IO and ABL.

Effects of Helicokinin-3 on Malpighian tubule function in the larval *Trichoplusia ni*.

Serna E, Gonzalez I, Solorio M, Kolosov D

Department of Biological Sciences, California State University San Marcos, San Marcos, California, USA.

The Malpighian tubules (MTs) and hindgut together act as the functional kidney in insects. MTs of caterpillars are complex and display regional heterogeneity in ion transport properties, ultrastructure and gene expression. The distal ileac plexus (DIP) is a region of lepidopteran MTs that is of particular interest because it switches from ion secretion to ion reabsorption in larvae raised on ion-rich diets. This switchover is a coordinated systemic response involving alterations in ion transport, water permeability and septate junction permeability. Kinins are a class of neuropeptide hormones with established importance in the regulation of ion and water transport in the MTs of insects. Recent studies indicate that lepidopteran kinin, helicokinin (HK), can coordinate changes in ion transport, water permeability and septate junction permeability in the MTs of larval lepidopterans when the tubules switch from ion secretion to ion reabsorption. Recent studies determined that there are three helicokinin isoforms, HK-1, -2 and -3 that originate from the same gene/transcript, but differ in their C-terminal amino acid residues. Although effects of HK-1 have been studied in detail, not much is known about HK-2 and HK-3 and their effects on ion transport, fluid secretion rate and septate junction permeability. This study was designed to investigate the effects of HK-3 on fluid secretion rate, ion transport and septate junction permeability in the DIP of larval Triochoplusia ni.

Acknowledgements: This research was supported by startup funds from CSUSM and internal research grant monies.

Voltage-gated calcium channels in epithelia of mosquitoes – effects of rearing environmental salinity in larvae and blood-feeding in adults.

S Farrell, N Ramirez, M Lopez-Sanchez, D Kolosov

Department of Biological Sciences, California State University San Marcos, San Marcos, California, USA.

Epithelia of animals often have to respond to rapid changes in environmental and systemic ion levels. Yellow fever mosquito *Aedes aegypti* has filter-feeding aquatic larval and terrestrial blood-feeding adult life stages. Recent work on the Malpighian tubules (MTs) of larval lepidopterans (caterpillars) demonstrated that the distal ileac plexus segment of this epithelium is capable of rapidly switching between ion secretion and reabsorption. Voltage-gated calcium channels were implicated in the regulation of ion transport in this epithelium, indicating that a functional CaV1 channel is necessary for constitutive K+ secretion observed in isolated preparations of lepidopteran MTs. Transcriptomic survey and pharmacological studies in the lab suggest that voltage-gated Ca2+ channel CaV1 is expressed in osmoregulatory epithelia of mosquitoes and may be implicated in salinity acclimation of larvae and blood feeding of adults. This study was designed to detect CaV1 mRNA and protein in the MTs and anal papillae of Aedes aegypti and monitor abundance of this voltage-gated ion channel in these tissues following salinity acclimation and blood-feeding.

Acknowledgements: This research was supported by startup funds from CSUSM and internal research grant monies.

The effects of Fusion on the larvae of *Chironomus riparius*: The search for alternatives to road salt.

S J Silver, J P Paluzzi, A Donini

Department of Biology, York University, Toronto, Ontario, Canada

Salinisation of freshwater is of global concern, and one of the sources of salinisation in colder climates is the use of NaCl to deice roadways. This salt enters freshwater creeks, rivers, and lakes as run-off, where it can have detrimental effects on freshwater animals. This study investigates the impact of an alternative de-icer, Fusion, a mixture of beet juice, NaCl and proprietary additives, on the larval midge *Chironomus riparius* ubiquitous in freshwaters of the world's northern hemisphere. When exposed to Fusion for ten days, the toxicity screening of this insect revealed that as Fusion concentration increased, the survival of *C. riparius* decreased. However, it was determined that larvae could tolerate 2 to 4% Fusion with minimal mortality, and these concentrations were used for subsequent experiments as high but sublethal doses. Physiological endpoints of osmoregulation were measured to determine if Fusion affects the osmoregulatory mechanisms of *C. riparius* larvae. Total body moisture was determined, and hemolymph ion concentrations were measured using ion-selective microelectrodes (ISME). The Na^+ concentration in the haemolymph was similar in larvae of *C. riparius* exposed to Fusion or freshwater control. Total body moisture was also similar in larvae exposed to Fusion and controls. Therefore, results suggest that *C. riparius* larvae exposed to relatively high but sublethal Fusion levels can effectively osmoregulate.

This research is supported by NSERC

Distribution of the adipokinetic hormone/corazonin-related peptide in the disease vector, *Aedes aegypti* and identification of key receptor residues necessary for ligand activation.

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The mosquito *Aedes aegypti* is a critical research model given its role as a major vector responsible for several arboviral diseases such as yellow fever, chikungunya and the Zika virus. Neuropeptides regulate several physiological processes in insects, including reproduction, feeding, diapause and metamorphosis. The adipokinetic hormone/corazonin-related peptide (ACP) is an insect neuropeptide structurally intermediate between adipokinetic hormone (AKH) and corazonin (CRZ). Despite the structural similarity and the close evolutionary relationship between ACP and AKH, their signalling systems function independently. Many studies have characterized the AKH and CRZ signalling systems within diverse insect species, and the most notable functions include energy substrate mobilization and responses to stress, respectively. In contrast, the function of the ACP signalling system remains unclear. In this study, we aimed to determine the specific regions of the ACP receptor (ACPr) most critical for ligand fidelity and specificity by creating ACPr chimera by singly replacing the complete or select highly conserved residues within the ACPr extracellular loops (ECL1, ECL2, and ECL3) and incorporating those from the AKH receptor. To date, heterologous functional assays have determined that three ACPr-ECL mutants receptors with complete replacement showed no response to either ACP or AKH. These results suggest the complete replacement of each extracellular loop is detrimental to ligand recognition. Further, we also aimed to map the distribution of ACP in the *A. aegypti* nervous system using immunohistochemistry. In adult mosquitoes, ACP immunostaining is localized in two pairs of lateral neurosecretory cells in the brain and 2-3 cells in the thoracic ganglia with ACP-immunoreactive axonal projections emanating within each abdominal ganglia. These results indicate that ACP might act as a neuromodulator and/or neurotransmitter.

This research was supported by an NSERC Discovery Grant and institutional laboratory start-up funds to J.P.P. The authors thank Dr. Jan Veenstra (University of Bordeaux) for providing the ACP antiserum used in this study.

The Influence of a Blood or Nectar Meal on Aquaporin Abundance in the Disease Vector Mosquito, *Aedes aegypti*.

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The disease vector mosquito, *Aedes aegypti* is responsible for the transmission of deadly arboviral diseases around the globe, particularly in subtropical and tropical regions. Adult female mosquitoes require a blood meal to obtain nutrients needed to produce mature, viable eggs. Both male and female *A. aegypti* acquire a nectar meal to provide necessary sugars and water. When females ingest a blood meal, the load is about three times the volume of their haemolymph and thereby poses an osmoregulatory challenge. Upon consumption of a nectar meal, both male and female *A. aegypti* experience an increase in haemolymph volume and solute concentration. The primary organ responsible for the production of urine in *A. aegypti* is the Malpighian tubules (MTs). There are five blind-ended tubules that are attached at their proximal end to midgut/hindgut junction. The principal and stellate cells of the MTs are responsible for the production of primary urine by the active secretion of ions driven by an apical V-type H+-ATPase in the principal cells. The movement of water in the MTs occurs via osmosis, following the movement of ions into the tubule lumen. The transport of water is made possible by the expression of aquaporin proteins in the MTs of *A. aegypti* (AaAQP). It has been shown that a blood meal taken by female mosquitoes and a nectar meal taken by both male and female mosquitoes, significantly increases the secretion of ions and water by the MTs. In this study, the AaAQP protein abundance and localization in the MTs of *A. aegypti* are assessed following blood meal engorgement by female and nectar feeding by male A. aegypti. Our results suggest that AaAQP1 and AaAQP4 abundance increases in the MTs of female *A. aegypti* at ~0.5hr and ~24hr post blood meal. Furthermore, AaAQP2 abundance appears to be altered by a sugar meal in male *A. aegypti*. The localization of AaAQP1 and AaAQP4 in the MTs is the subject of continuing work.

This research was supported by NSERC.

Elucidating the role of the V-type H+ ATPase in CAPA-mediated inhibition in *Aedes aegypti* Malpighian tubules.

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Active ion transport in Aedes aegypti Malpighian tubules (MTs) is driven by the V-type H+-ATPase (VA), serving as the primary energizer for transepithelial secretion of electrolytes and water. Produced in the nervous system, the anti-diuretic peptide, CAPA, inhibits fluid secretion of MTs stimulated by select diuretic factors, serotonin (5HT) and calcitonin-related diuretic hormone (DH31) through the NOS/cGMP/PKG pathway, however, the downstream cellular targets remains unclear. Given the predominant role of the VA in fluid secretion, the objectives of this study were to examine the involvement of the VA in CAPA-mediated inhibition. Bafilomycin, a VA inhibitor, was found to inhibit fluid secretion stimulated by 5HT and DH31, whilst having no inhibitory action on MTs stimulated with the corticotropin-releasing factor-related diuretic hormone (DH44). CAPA and bafilomycin treatment led to alkalization of the secreted fluid suggesting inhibition of the apical VA, which may lead to constrained entry of cations across the MT membrane. Additionally, VA activity was increased in adult female MTs treated with DH31, whereas CAPA treatment reduced VA activity, comparable to saline levels. To determine whether CAPA causes V1 dissociation from the VA holoenzyme, cytosolic and membrane protein fractions were isolated from DH31- and CAPA-incubated MTs. V1 protein expression was found to be higher in the membrane fractions of DH31-incubated MTs while higher levels were seen in the cytosolic fractions of CAPA-treated tubules. Lastly, immunohistochemical techniques revealed both V1 and Vo localization exclusively in the apical membrane of DH31-incubated MTs, whereas V1 immunoreactivity was observed in both the apical membrane and cytosolic portion of CAPA-incubated MTs. These results suggest a novel mechanism for CAPA inhibition, inhibiting VA activity to hinder fluid secretion.

Acknowledgements: Supported by an Early Research Award from the Ontario Ministry of Research & Innovation and an NSERC Discovery Grant (JPP) as well as NSERC CGS, Vernon Stong, and Carswell Scholarships in Science (FS)

Expression profiling and characterization of CCHamide2 neuropeptide and its G-protein coupled receptor in the yellow fever mosquito, *Aedes aegypti*.

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As a widely distributed anthropophilic mosquito species, *Aedes aegypti* is able to transmit various pathogens leading to human diseases. Studying the neuroendocrine system of *A. aegypti* allows us to understand mosquito physiology better and provides insights into mammalian neuroendocrinology based on the comparative approach. The neuropeptide CCHamide2 (CCHa2) and its associated G-protein coupled receptor (CCHa2R) were recently identified across insects. A CCHa2R mammalian homolog, Bombesin receptor subtype-3, was localized within the insulin-producing beta cells of the endocrine pancreas. However, expression profiles and physiological role of CCHa2 and CCHa2R in A. aegypti remain unclear. This research aims to examine, quantify and localize expression of CCHa2 and CCHa2R and elucidate its physiological functions in the yellow fever mosquito. To date, RT-qPCR demonstrated transcript abundance of CCHa2 and CCHa2R change throughout developmental stages with the highest expression in one day old male adult and late-stage pupa, respectively. Different abundance of CCHa2 transcript is also observed in mosquito adults of each sex. Results are somewhat consistent with findings in *Drosophila melanogaster*, except for distinct transcript abundance of CCHa2 detected in fly adults of each sex. Previous studies reported Drosophila CCHa2 transcript enriched in the gut and CCHa2R highly expressed in the nervous system including insulin-producing cells in the fly brain. Similar methods are being used to quantitate the tissue and organ-specific transcript abundance in adult mosquitoes. Further, immunohistochemistry and in situ hybridization will be utilized to localize cell-specific expression of CCHa2 and its receptor CCHa2R. Future research also aims to use a heterologous expression system to confirm the specificity and sensitivity of receptor (i.e. CCHa2R) activation by the CCHa2 peptidergic ligand. Finally, based on preliminary expression data of CCHa2 and CCHa2R, various bioassays and reverse genetic approaches will be employed to elucidate the physiological roles of CCHa2 in this important human-disease vector.

Insights into the physiological significance and bioproperties of underwater silks spun by chironomid midges.

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Salivary glands of the aquatic midge, *Chironomus* are dedicated for the continuous synthesis of secretory proteins that are vital for physiological processes. Of these, midges use the viscous silk secretions to bind substratum particles and spin underwater housing-cum-feeding nests. Unlike other silk-spinning insects, a notable advantage offered by chironomid midges is the harvestation of silk without killing the animal. However, midges are an underappreciated model in this respect and owing to the huge gap in this research area, the nature and properties of midge silk remained untested. Here, we present the first report on the molecular and biophysical characterisation of aquatic silk from a tropical midge species, *Chironomus ramosus*. As a prerequisite to our investigation and to mimic larval nest building behaviour in the laboratory, we chose beach sand as a suitable inert and non-toxic substratum material. Based on gene expression analysis and chromosomal puffing patterns of the Balbiani rings I and II as marker loci for active transcription of silk proteins, we confirmed that the early- to late-third larval instars were prolific silk producers. Mechanical measurements and microscopy-based methods revealed the morphological features and exceptional tensile performance of the nest composites. Furthermore, we have optimized a futuristic commercial-scale technique of silk extraction from the larvae in a non-violent way. A combination of biochemical and biophysical techniques provided critical insights on the structural and biomechanical characteristics of midge silk that potentially contribute to its multi-functionalization. In summary, we shed light on the unique and superior properties of *Chironomus* silk as a novel water-borne biopolymer which can be exploited for commercial and biotechnological applications. This study is also particularly relevant to the 'Peace silk' industry, that promotes the implementation of cruelty-free methods for silk harvestation from animals. This study was supported by the DBT-BioCARe research grant awarded to LT and the ISF-UGC-Indo-Israel research grant awarded to BBN.

Identifying and characterizing an IMD candidate in *Rhodnius prolixus* using 3D modeling, phylogenetic conservation, and gene knockdown approaches.

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Invertebrate innate immune signaling pathways are highly conserved among insects, and serve to amplify and regulate immune responses towards parasites and pathogens. One of these pathways is the Immune deficiency (IMD) pathway that, in most insects, detects Gram-negative bacteria using receptors that interact with the intracellular protein IMD. While this pathway is highly conserved in holometabolous insects, recent studies on many hemimetabolous insects suggest the IMD pathway is variable in easily identified gene members. In some insects, including *Rhodnius prolixus*, a hemipteran vector of the human parasite *Trypanosoma cruzi*, the IMD pathway was assumed to be absent or non-functional as many key genes in this pathway were reported as absent, including IMD itself. Many of the 'missing' elements have since been identified and are associated with the control of immune responses to Gram-negative bacteria. Only IMD and Kenny have not been identified yet. We created a tridimensional model of a candidate IMD gene (rpIMD) that has a weak resemblance to the honeybee IMD. Despite poor sequence homology and the absence of specific domains, this gene is predicted to form a Death Domain typically found in all insect IMD proteins. We also detected in rpIMD a cRHIM, that is required for the interaction of IMD proteins with receptor molecules. Despite these conserved properties, silencing of rpIMD using multiple RNAi approaches failed to diminish immune responses towards Gram-negative bacteria, in contrast to results obtained after silencing other IMD pathway genes. There is, however, a strong upregulation of rpIMD in rpIMD silenced insects exposed to bacteria, suggesting its participation in regulating immune responses. The implications of these results are evaluated in terms of the evolution of the IMD pathway, and we are looking forward to discussing your interpretation of our results.

Voltage-gated ion channels in animal epithelia – what gives?

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Epithelia are multifunctional tissues of metazoans used for directional ion and water transport. Voltage-gated ion channels are expressed in epithelia of many animals, including osmoregulatory epithelia of insects. Recent bioinformatics- and laboratory-based work has implicated voltage-gated ion channels in the regulation of ion transport in the Malpighian tubules of insects. Our laboratory at California State University San Marcos is using several animal models to unveil how voltage-gated ion channels function in animal epithelia. This talk is designed to increase exposure of our new lab and its members and to describe studies in progress in our lab.

Acknowledgements: This research was supported by startup funds from CSUSM and internal research grant monies.

The effects of nitric oxide on the cardiovascular system and immune response of the mealworm.

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We are interested in understanding how interacting physiological systems facilitate the success of pest insects. In this study, we explored the effects of the unconventional signaling molecule, nitric oxide (NO), on the *Tenebrio molitor* immune and cardiovascular system, and how these systems interact with one another to fend off pathogens. NO production was induced by applying L-arginine, a precursor to the NO-L-arginine pathway, to the insect heart. MAHMA NONOate, a nitric oxide donor was also tested. In both cases, there was an observed decrease in heart rate. A nitric oxide synthase (NOS) inhibitor (L-NAME), increases heart rate dose-dependently. Utilizing immunohistochemical analysis, we have explored the activation of NOS together with the JNK and IMD immune pathways in these insects when challenged with abiotic microbeads, or when infected with gram-negative bacteria *Escherichia coli*. Our study showed that when abiotic and/or biotic stressors infect T. molitor, there was a significant increase in NOS production suggesting NO has a role in insect immunity within these insects. Additionally, when NOS was inhibited in hemocytes of T. molitor, there was a significant decrease of JNK and Relish expression in cells suggesting NO has a role in regulating immune protein production. Hemocytes inoculated with green fluorescent beads alone, and in conjunction with E. coli revealed that when cells were exposed to the biotic stressor, there was an increased amount of beads engulfed by hemocytes. Peptidoglycan-related signaling pathways, like the IMD and JNK pathways, may therefore influence phagocytic ability of hemocytes.

Discovery of the elusive *Drosophila* ion transport peptide (ITP) receptor.

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Insects have evolved a variety of neurohormones that enable them to maintain their osmotic and ionic balances under different environmental conditions. While the identities and functions of various insect diuretic hormones have been well-established, studies characterizing the presence of an anti-diuretic signaling system that is conserved across most insects are still lacking. To address this, here we identify and characterize the receptor for Ion Transport Peptide (ITP) which has for long been considered the insect anti-diuretic hormone. Extensive anatomical mapping of the ITP receptor (ITPR) using a newly-generated GAL4 line reveals that it is broadly expressed in larvae and adults of *Drosophila*. Importantly, ITPR is expressed in Malpighian tubules (MTs) and the rectal pads, tissues that are associated with insect osmo/ionoregulation. Consistent with this expression, recombinant Drosophila ITP inhibits basal as well as Leucokinin stimulated MT secretion. In addition, this effect is mediated via ITPR as MT-specific knockdown of ITPR abolishes the decreased secretion rates observed following application of recombinant ITP. Insect ITP, crustacean hyperglycemic hormone (CHH) and moult inhibiting hormone (MIH) are evolutionary related and thus part of a large peptide superfamily. Members of peptide superfamilies often have conserved functions. Hence, besides osmoregulation, ITP signaling could also regulate glucose homeostasis as ITPR is highly expressed in neurons producing insulin and glucagon-like adipokinetic hormone. Moreover, knockdown of either ITP or ITPR results in developmental lethality implicating a role in moulting and/or development. Since our phylogenetic analysis reveals that ITPR is conserved throughout arthropods, this work suggests that ITP signaling may serve in anti-diuresis in other arthropods.

Characterization of RYamide Neuropeptide and Its Receptor in the Human Disease Vector, *Aedes aegypti*.

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RYamides are a family of neuropeptides that were recently discovered among insects. About a decade ago, dRYamide-1 and -2 were found to be endogenous ligands to the *Drosophila* receptor CG5811, a receptor initially shown to react to the mammalian neuropeptide Y (NPY). Although it was deduced that CG5811 was not evolutionary related to NPY receptors, further research has shown similarities between RYamides and mammalian NPY regarding feeding behaviour. In larval and adult blowfly, *Phormia regina*, injection of RYamides causes the suppression of feeding motivation when the insect was presented with solutions containing sucrose. In the silkworm *Bombyx mori*, RYamides can inhibit hindgut contractions, which was proposed to be involved in feeding termination. Furthermore, RYamide receptor expression in the hindgut has been identified in various *Drosophila* species, and in *B. mori*. Besides contractability, the hindgut reabsorbs water and important ions into the hemolymph to aid in both digestion and osmoregulation. Preliminary research on adult *Aedes aegypti* using real-time PCR has indicated a proposed RYamide receptor having transcript expression in the hindgut where further research will aim to confirm the authenticity of the receptor, and its specificity to RYamides. In addition, immunohistochemistry results have suggested the presence of RYamides in the terminal abdominal ganglion, which innervates the hindgut. Functionally, these neuropeptides will be tested for their regulation of the hindgut by utilizing scanning ion-selective electrode technique, and hopefully RNAi in the future. Ultimately, my research aims to elucidate the regulatory function that RYamides have on the hindgut in Aedes aegypti.

This research is supported by York University and NSERC.

Identification of a tachykinin receptor and its implication in metabolism in *Rhodnius prolixus*, a Chagas disease vector.

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Neuropeptides and their receptors are fundamentally important for regulating many physiological and behavioural processes in insects, including in the Chagas disease vector *Rhodnius prolixus*. In this work, we have identified, cloned and sequenced the tachykinin receptor from *R. prolixus* (Rhopr-TKR) fifth instars. The receptor is a G protein-coupled receptor belonging to family A. The total length of the open reading frame of cDNA of Rhopr-TKR is 1014 bp, which translates into a receptor of 338 amino acids with seven transmembrane domains. Sequence analyses show high similarity and identity between Rhopr-TKR and other cloned invertebrate and vertebrate tachykinin receptors. The transcript expression level of Rhopr-TKR is highest in the central nervous system (CNS), followed by the fat body, an interchanging center remotely integrating with the CNS to regulate nutritional signals, suggesting a role of Rhopr-TKR in metabolism. Fluorescent in-situ hybridization for the Rhopr-TKR transcript shows a signal in a group of 4 bilaterally paired neurons in the protocerebrum of the brain. Using RNA interference, we generated insects with transcript knockdown of Rhopr-TKR to examine the Rhopr-TK signaling pathway's role in lipids and carbohydrates metabolism during the first 24 h after a blood meal. Knockdown of Rhopr-TKR led to a decrease in the size of the blood meal and a significant increase in hemolymph carbohydrate and lipid levels. Further investigation revealed that those insects in which the Rhopr-TKR transcript had been knocked down had decreased transcripts levels for the *R. prolixus* insulin-like peptide (Rhopr-ILP), R. prolixus insulin-like growth factor (Rhopr-IGF) and *R. prolixus* insulin receptor (Rhopr-InR) in both the CNS and fat body. Taken together, these findings suggest that Rhopr-TKR interacts with the insulin-signaling pathway and is involved in regulating lipid and carbohydrate metabolism and mobilization.

Acknowledgements: This work was supported by an NSERC Discovery Grant to ABL.

Identifying the glycosylation site on *Drosophila melanogaster* ion transport peptide and its potential physiological role.

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The excretory system of the fruit fly, *Drosophila melanogaster*, consists of the hindgut and Malpighian tubules (MTs) and functions to maintain ion and water balance. The MTs are under endocrine control to maintain haemolymph homeostasis and prevent desiccation. The ion transport peptide (ITP) was first identified as an anti-diuretic hormone to stimulate fluid reabsorption of the locust hindgut. In silico studies have shown a predicted glycosylation site in *Drosophila* ITP, which provided evidence for a potential role of glycosylation in neuropeptide hormones. In the present study, we identified the predicted glycosylation site of *Drosophila* ITP and examined its physiological role on the MTs. To validate the predicted glycosylation site, constructs of wild-type ITP (glycosylated) and mutated ITP (non-glycosylated) were created and expressed in a heterologous system using mammalian cell lines. We then collected protein samples from the cell culture to conduct western blot analyses. The results revealed that glycosylation occurs at an asparagine residue (position 13), which was only present in the wild-type ITP and not the mutated ITP. We then assessed the physiological role of ITP glycosylation using the Ramsay assay to measure the fluid secretion rate of isolated *D. melanogaster* MTs stimulated with a kinin diuretic hormone. Studies are ongoing to investigate the physiological relevance of ITP glycosylation and its role as an anti-diuretic hormone.