Short neuropeptide “F” and the nutritional and reproductive status of the red imported fire ant colony

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Outline

- Invasive species with high reproductive output
- Neuropeptide control of nutrition and reproduction: the sNPF system as candidate
- Molecular, biochemical characterization of the fire ant sNPF receptor:

- Queens: a link between nutrition and reproduction

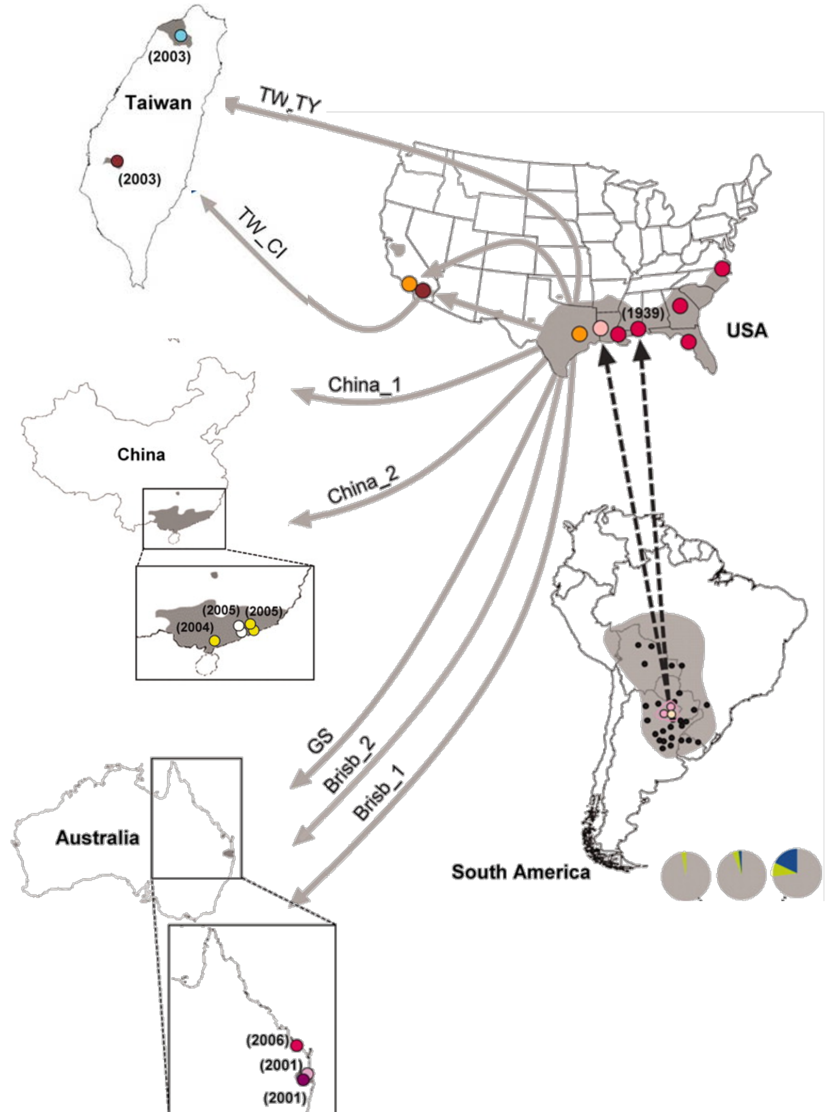
- Workers: The sNPF receptor in worker division of labor and sensing nutritional status

- **My work: Deorphanization of the sNPF receptor**

Solenopsis invicta Buren
The red imported fire ant

- Red imported fire ant was introduced to the USA from South America in 1930 and rapidly spread throughout the southern United States

- Recently to California and other regions of the world Australia, New Zealand, Taiwan, Hong Kong, Macao and Mainland China



Ascunce et al. 2011. Science. 331: 1066-1068

Solenopsis invicta Buren
The red imported fire ant

Mature mounds reach ~35 cm (1 foot) in height and have no openings except just before and after a mating flight

Photo: Texas imported fire ant research and management project.

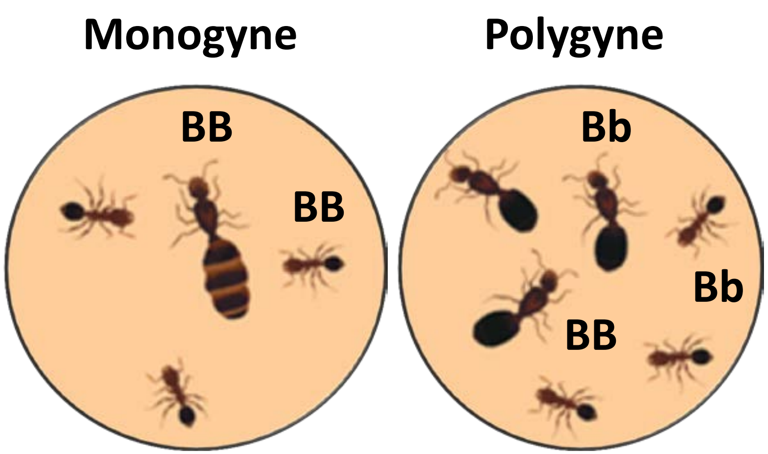
- serious agricultural, medical, and urban pest
- damage to property in the United States alone is greater than $6 billion annually (Lard et al. 2006)

Vinson SB. 1997. American Entomologist. *43: 23-39.*

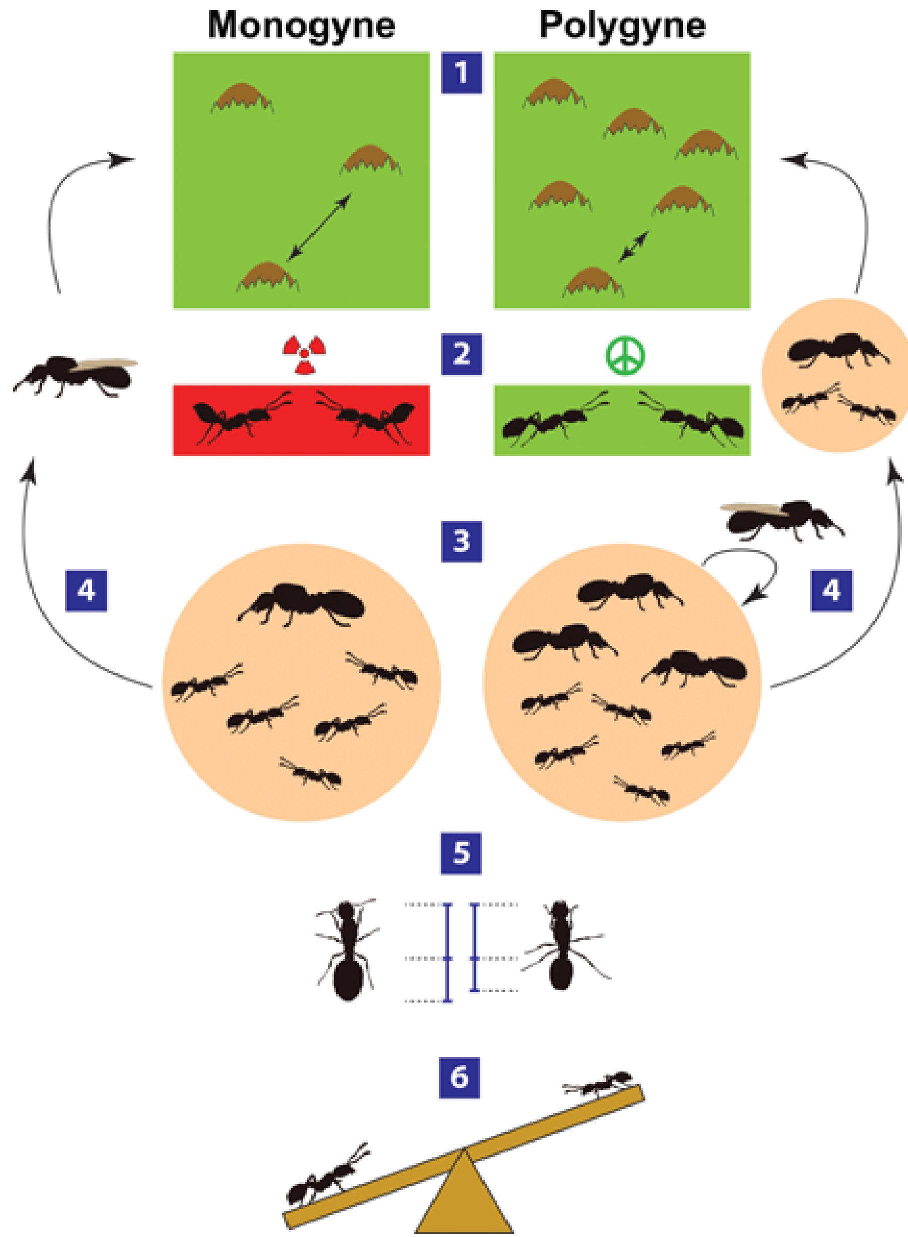
The Red Imported fire ant social forms

The most important organizing feature of an ant colony is the number of queens

- Monogynous colonies: headed by a single queen
- Polygynous colonies: headed by multiple queens



Completely associated with variation at the marker gene Gp-9, which has two alleles, 'B' and 'b'



Huang and Wang. 2013.Bioessay. 36: 200-208

Fire ant colony organization

Reproductive

- Queen
- Male

Sterile

- Workers

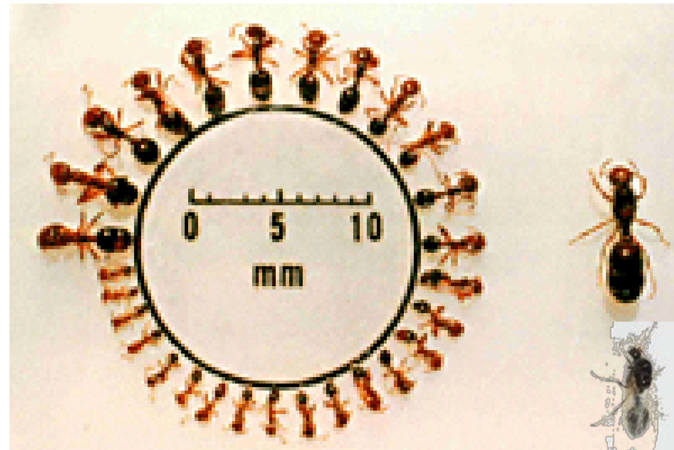
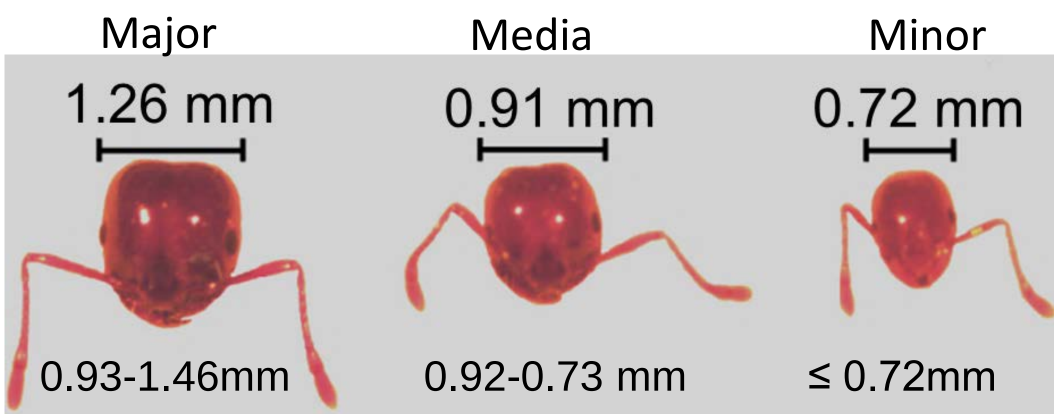


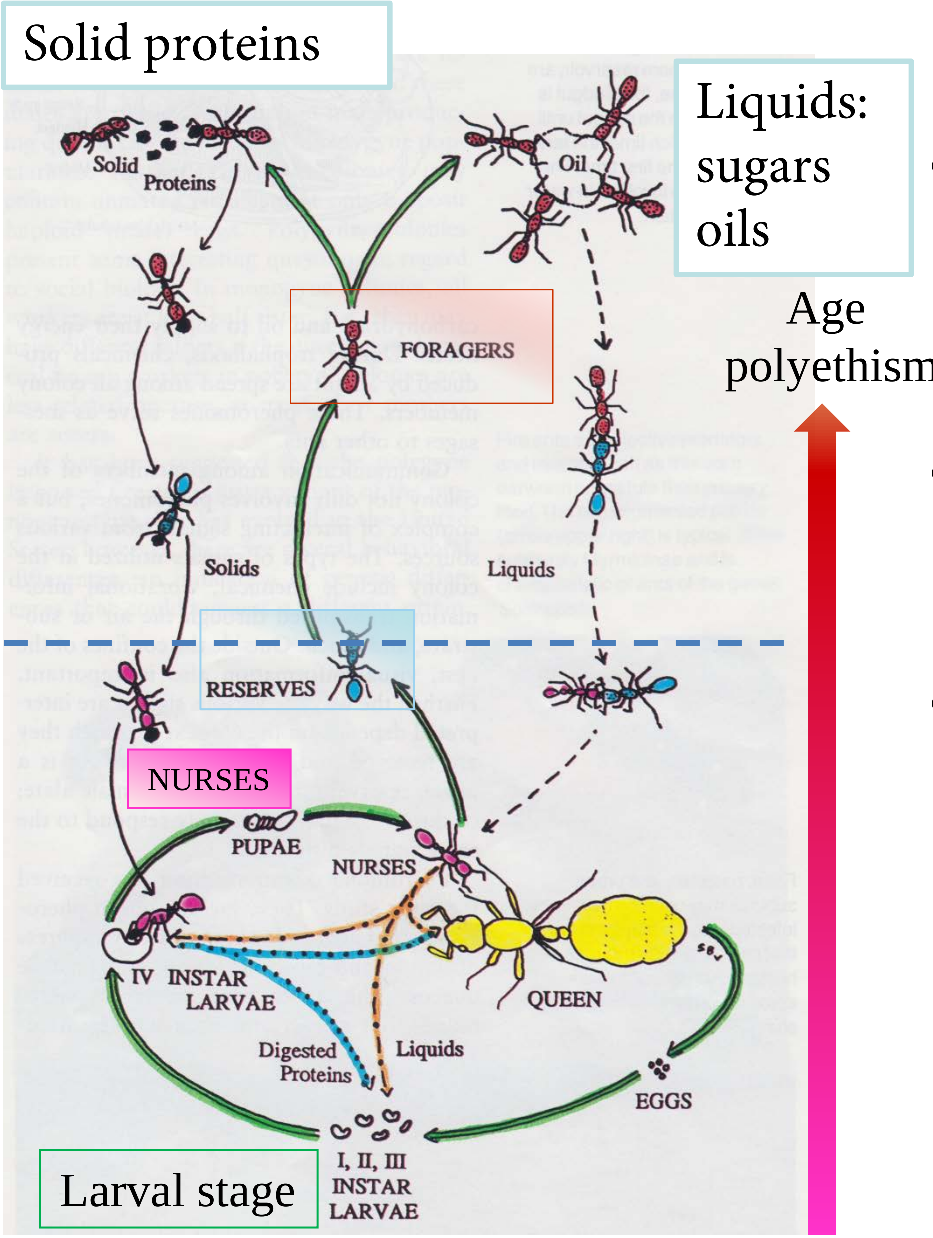
Photo by Sanford Porter



According to task performance workers can be divided into nurses, foragers and reserves

Castillo P., Pietrantonio P.V. (2013) PLoS ONE 8: e83966

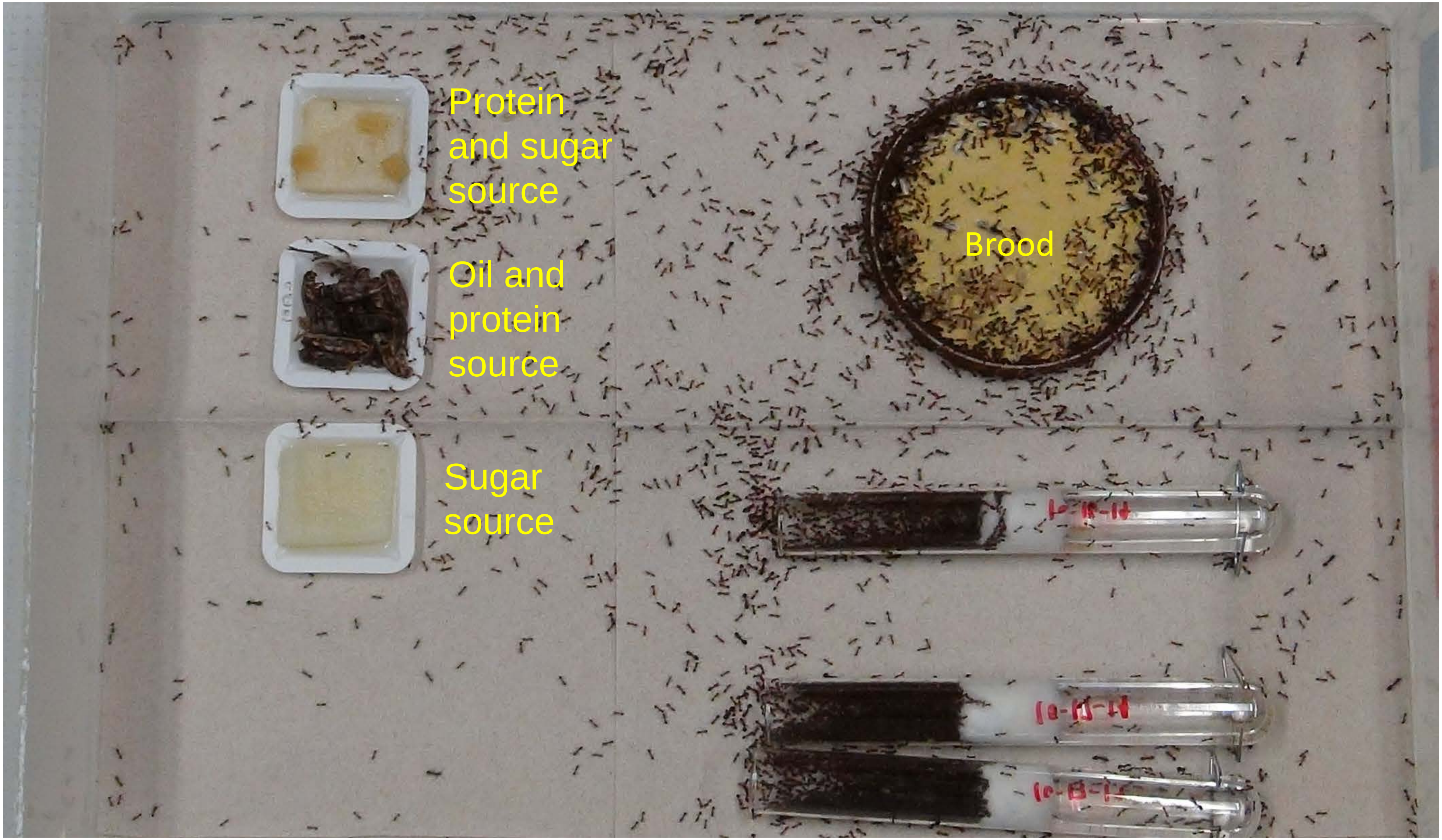
Food flow in the fire ant colony



Vinson SB. 1997. American Entomologist. *43: 23-39.*

- All the members of the colony feed by sharing the liquid food via trophallaxis
- Workers forage for liquid and solid foods
- Only 4th instar larvae can feed upon solid foods and digest protein for the colony

Fire ant colony in the laboratory



Neuropeptides signaling system controls physiology

- Neuropeptides control physiological functions of insects such as feeding, locomotion, development, and reproduction
- Social organization in insects highlights the challenge of understanding the physiological functions of a “superorganism”: what genes control social behavior?
- Neuropeptide Y: the first feeding-stimulatory neuropeptide discovered in mammals (Stanley and Leibowitz 1985)
- In insects, long and short NPF have been identified as orthologues of mammalian NPY
- Insect NPF peptides are ~36 amino acid residues in length (“long”)
- Insect sNPF peptides are 6-11 amino acid residues (“short”)

Candidate gene approach for the analysis of genes involved in regulation of nutritional status

sNPF peptides have been identified in several insect species, the Colorado potato beetle, fruitfly, locust etc (Spittaels et al. 1996; Broeck 2001; Schoofs et al. 2001)

In fruit fly sNPF peptide controls food intake and regulates body size (Lee et al. 2004)

sNPF receptor has been first identified from *Drosophila* (Mertens et al. 2002)

What is the significance of sNPF in social insects and in colony nutritional status?

First GPCR cloned from the fire ant and first demonstration of a role in nutritional status

The paper was cited by Gene Robinson's group ' the expression response of NPY-family receptors to nutritional manipulations has been studied in only one other invertebrate species, the fire ant *Solenopsis invicta'* Ament et al. 2011

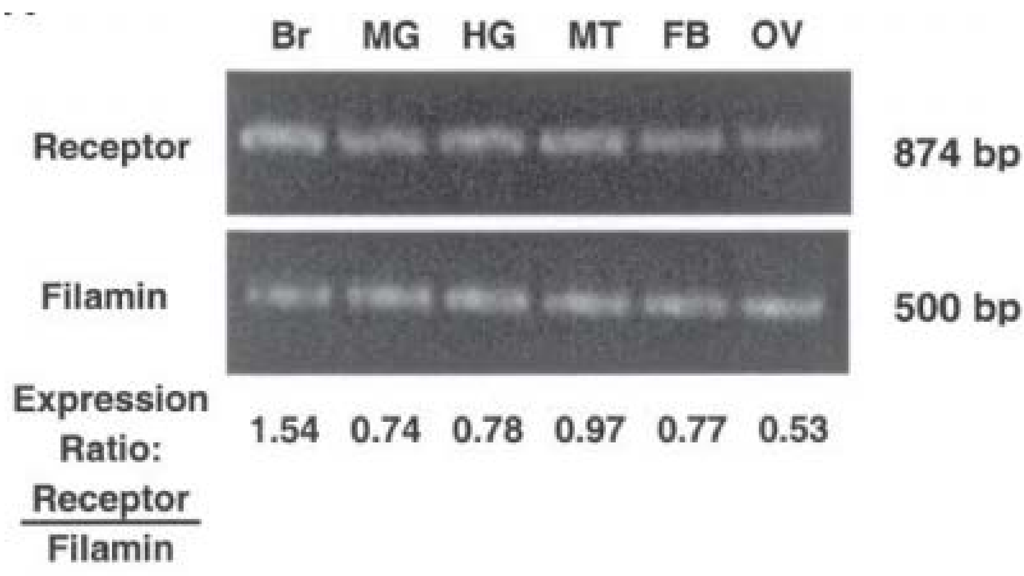
Archives of Insect Biochemistry and Physiology 61:195–208 (2006)

The Short Neuropeptide F-Like Receptor From the Red Imported Fire Ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae)

Mei-Er Chen and Patricia V. Pietrantonio*

In invertebrates, neuropeptide F (NPF) peptides share structural similarity with vertebrate neuropeptide Y, which regulates food consumption, circadian rhythms, anxiety, and other physiological processes. The insect neuropeptide F receptors belong to the G protein-coupled receptor (GPCR) rhodopsin family. We have cloned the fire ant putative short NPF receptor using PCR and RACE methods. The complete 2,185-bp cDNA encodes a 387-residue protein with a predicted GPCR seven transmembrane region structure. We propose that the sequence of the honey bee short NPF receptor, which has not yet been annotated, encodes a protein of 393 residues. In fire ant mated queens, receptor transcripts were detected in the brain, midgut, hindgut, Malpighian tubules, fat body, and ovaries. The highest transcriptional expression was found in the brain. The downregulation of the fire ant short NPF receptor transcriptional expression in the brain with starvation suggests that the short NPF signal transduction cascade may play a role in feeding regulation in fire ant mated queens. Arch. Insect Biochem. Physiol. 61:195–208, 2006.

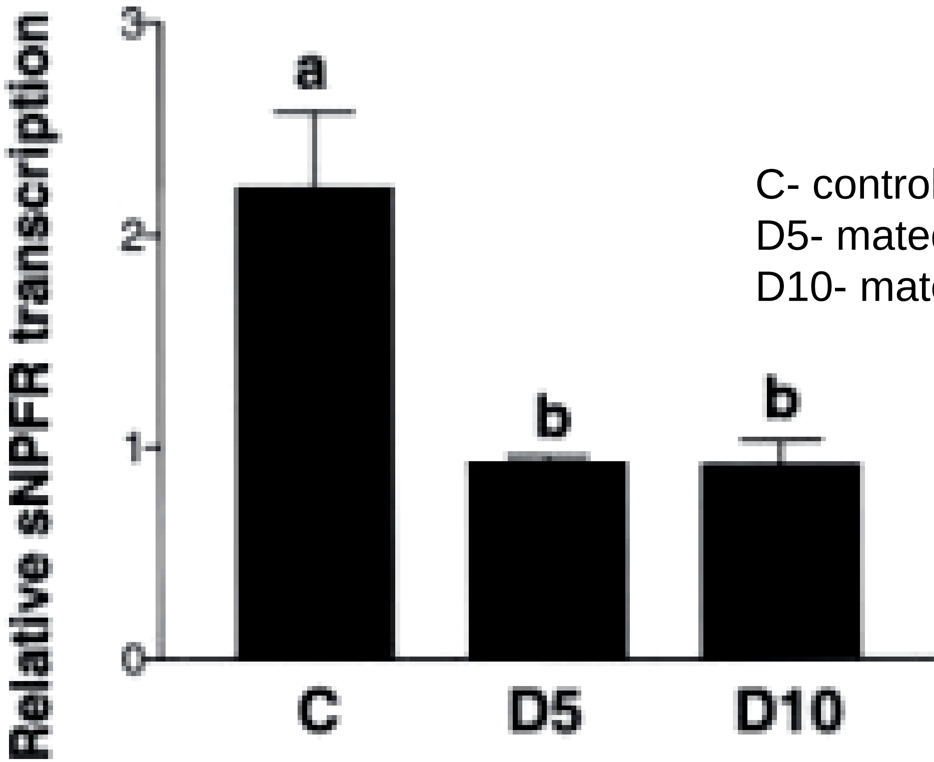
Short neuropeptide F receptor transcript was identified in various organs of the fire ant queen



Chen and Pietrantonio. 2006 .Arch Insect Biochem Physiol*. 61: 195-208*.

sNPF receptor transcript relative abundance at the central level is sensitive to changes in nutritional status

sNPF receptor transcript decreased in the starved fire ant mated queen's brain (semi-RT PCR)



C- control (fed mated queen)
D5- mated queens starved for 5 days
D10- mated queens starved for 10 days

*Chen and Pietrantonio. 2006 .*Arch Insect Biochem Physiol*. 61: 195-208*.

Subsequent research supports sNPF signaling system controls feeding behavior in insects: negative and positive correlations found with fed status

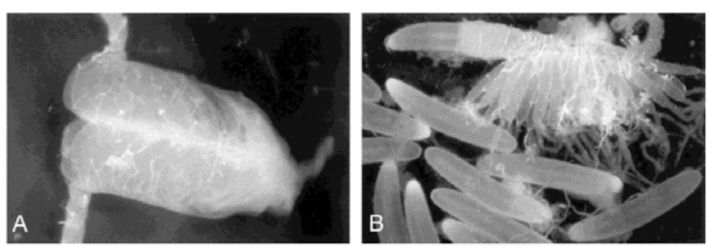
- In *Bombyx mori* larvae sNPF1, sNPF2 and sNPFR transcripts decrease upon starvation (Nagata et al. 2012)
- Colorado potato beetles are devoid of sNPF peptides during diapause, a period of starvation (Huybrechts et al. 2004)
- *Schistocerca gregaria* sNPF signaling plays inhibitory role in feeding (Dillen et al. 2013; 2014)

- In contrast, sNPFR is overexpressed in starved fruit flies, and cockroaches (Root et al. 2011; Mikani et al. 2012)

sNPF signaling system: linking reproductive control and nutritional status

- Copulation and competition with the potential mates need enormous energy
- Reproduction requires substantial metabolic energy for synthesis of vitellogenin for oocyte maturation
- Potential gonadotropin in locust

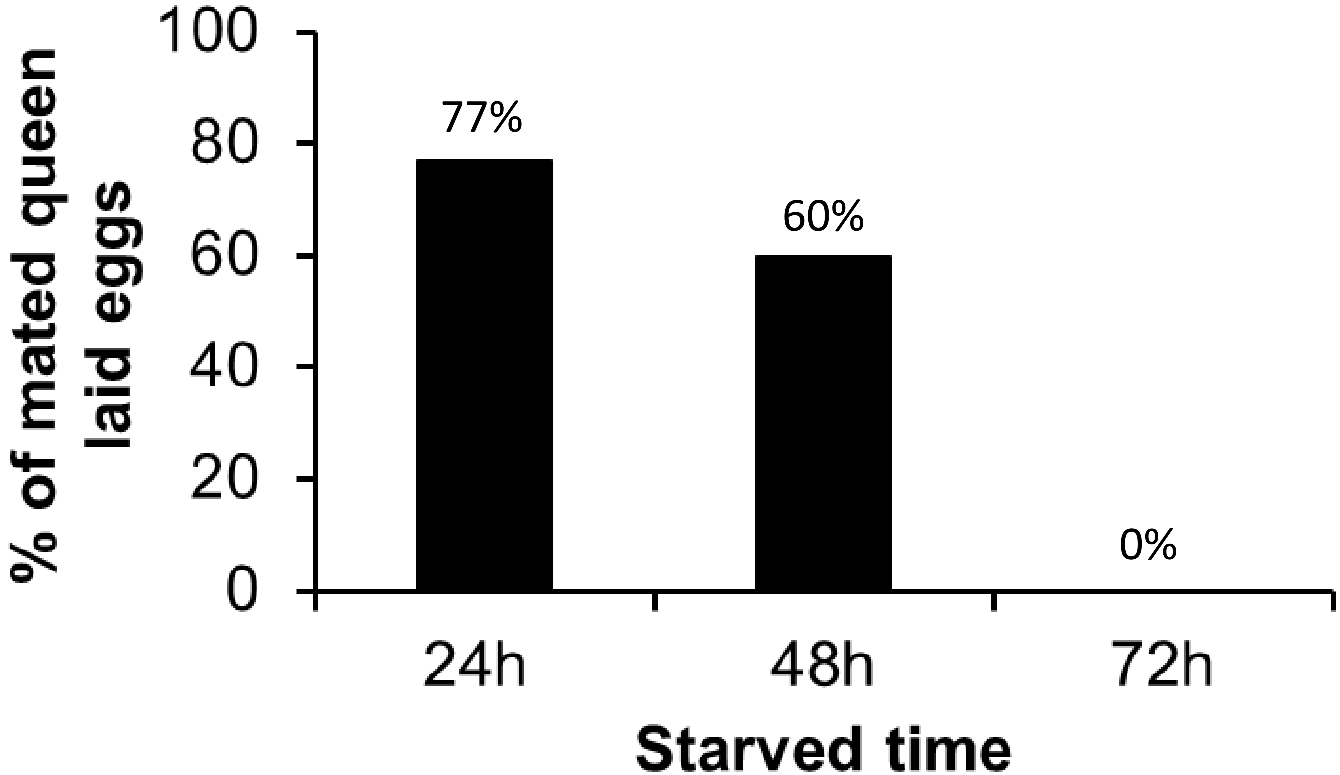
Ovary of *Locusta migratoria*



(De Loof et al., 2001)

Is sNPF signaling related to fire ant ovary development and reproduction?

Starvation causes the reduction of egg laying in the mated queens



*Chen and Pietrantonio. 2006 .*Arch Insect Biochem Physiol*. 61: 195-208*.

First demonstration of sNPF receptor protein in the ovary and brain of fire ant: sNPF peptide as potential brain-ovary neurohormone

Lu and Pietrantonio *BMC Neuroscience* 2011, **12**:57
http://www.biomedcentral.com/1471-2202/12/57

BMC Neuroscience

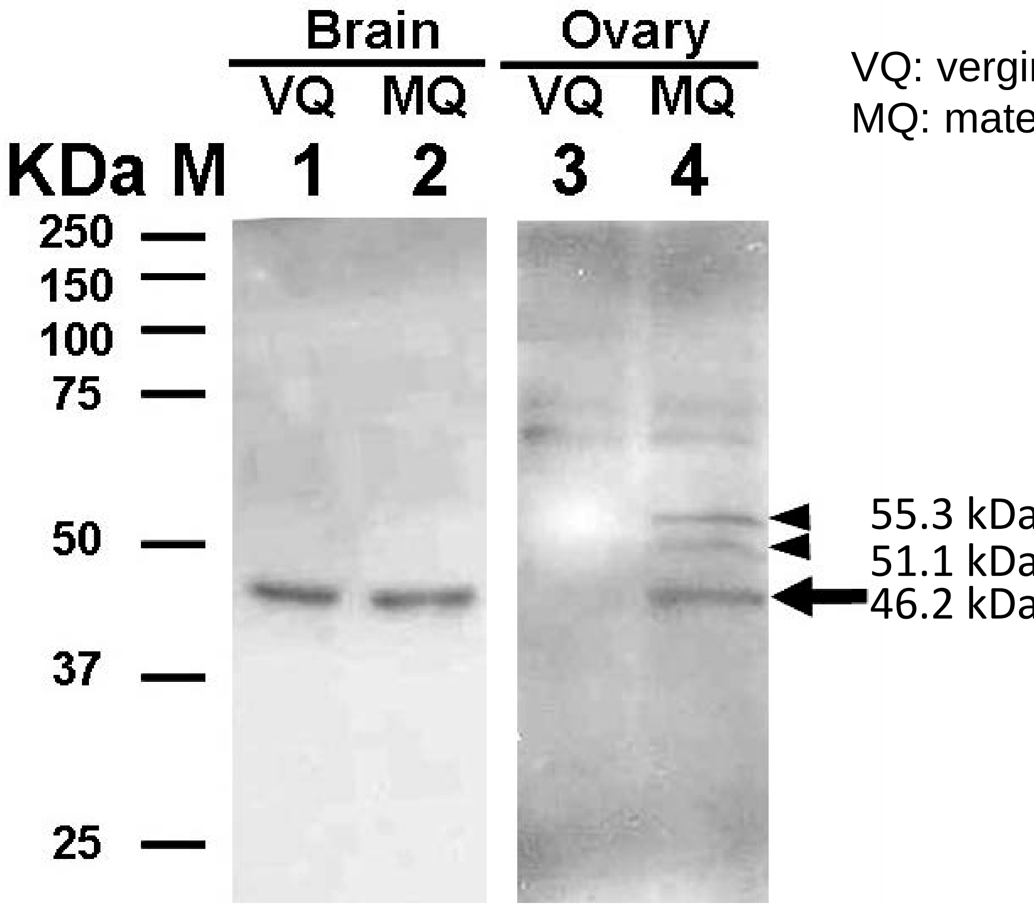
RESEARCH ARTICLE Open Access

Immunolocalization of the short neuropeptide F receptor in queen brains and ovaries of the red imported fire ant (*Solenopsis invicta* Buren)

Hsiao-Ling Lu and Patricia V Pietrantonio*

Conclusions: The analysis of sNPF receptor immunolocalization shows that the sNPF signaling cascade may be involved in diverse functions, and the sNPF peptide(s) may act in the brain as neurotransmitter(s) or neuromodulator(s), and in the ovaries as neurohormone(s). To our knowledge, this is the first report of the cellular localization of a sNPF receptor on the brain and ovaries of adult insects.

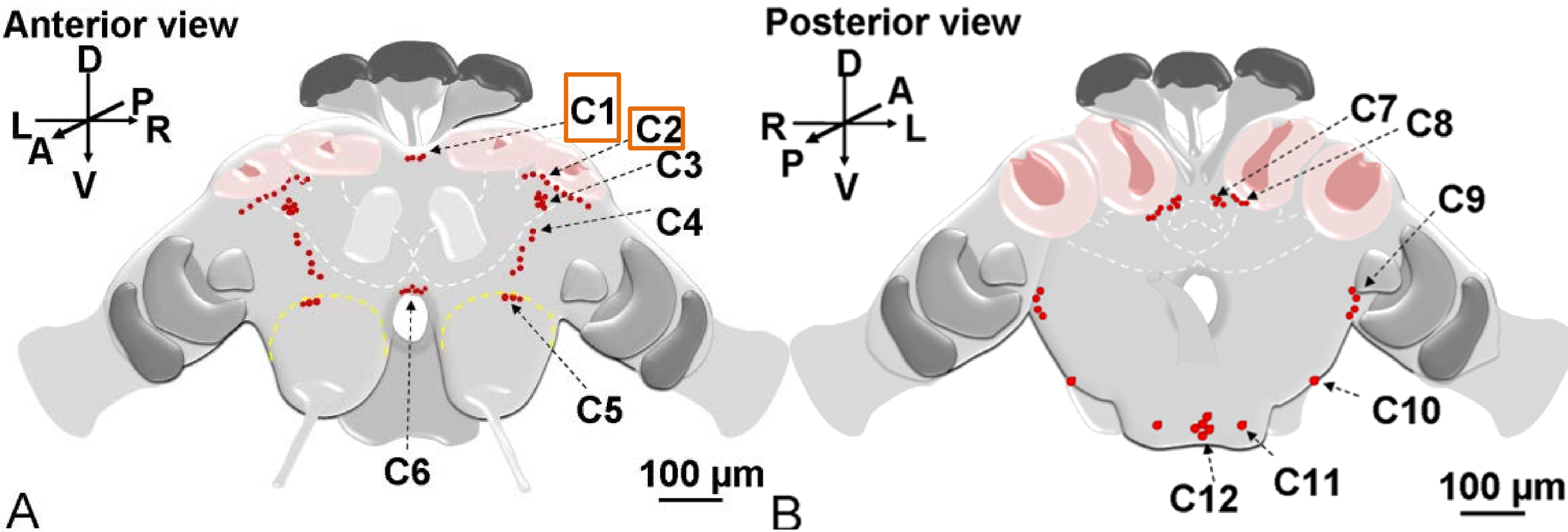
Detection of the sNPFR protein in fire ant queen brain and ovary



VQ: vergin queen
MQ: mated queen

Lu and Pietrantonio. 2011. *BMC Neuroscience* 12:57

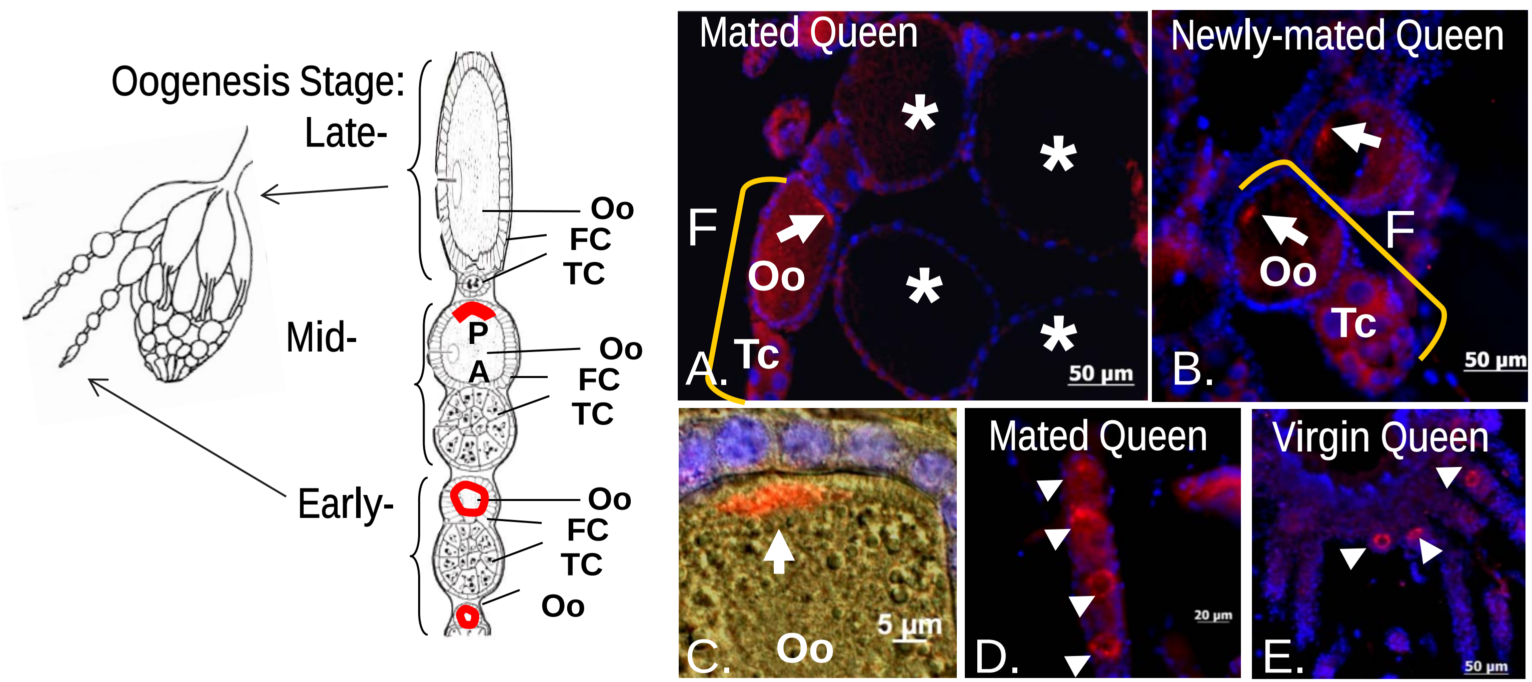
Immunolocalization of sNPFR in the fire ant queen brain



Lu and Pietrantonio *BMC Neuroscience* 2011 **12**:57

Localization of sNPFR protein in the mated queen ovary

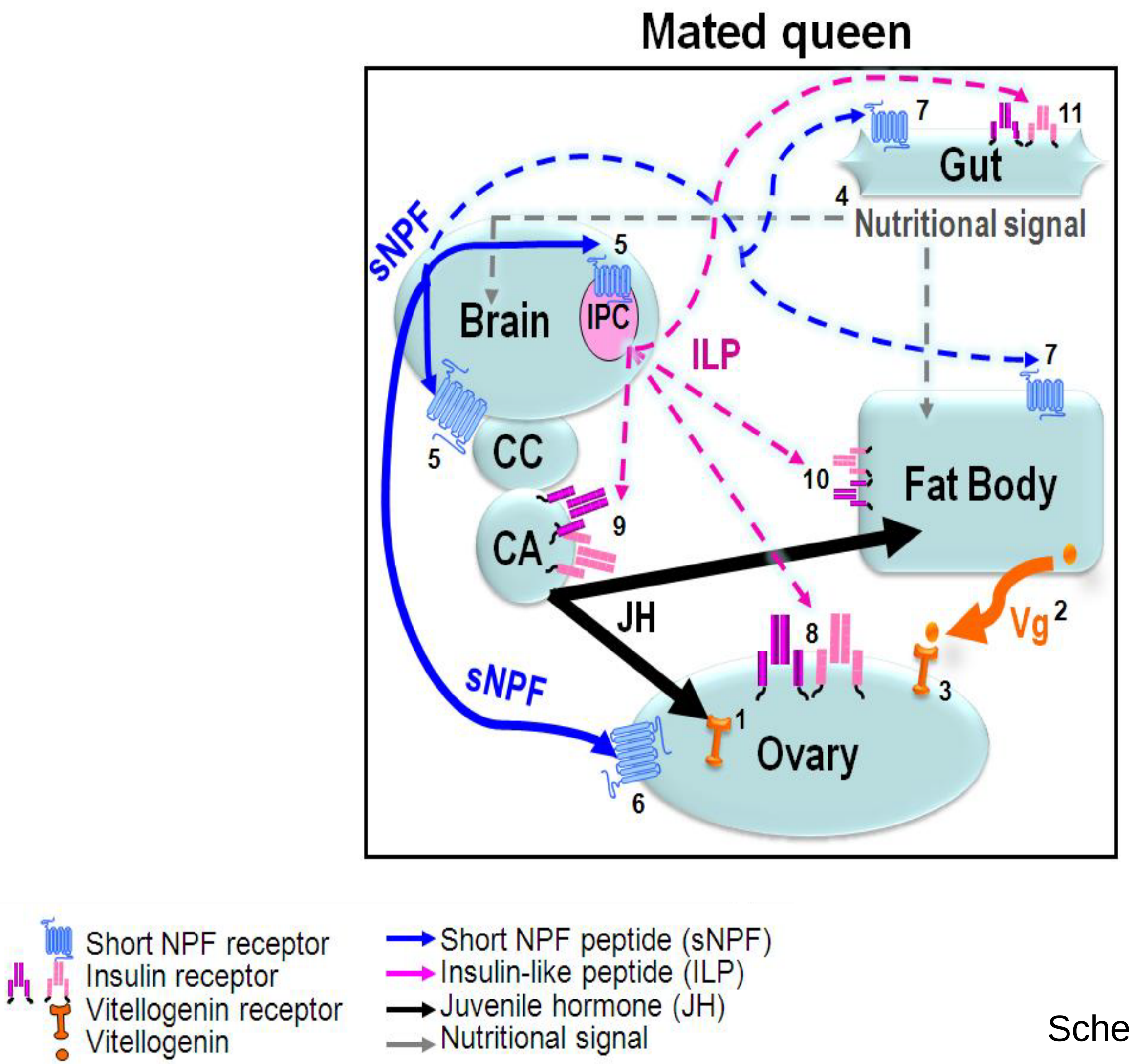
- Receptor signals were detected in the posterior end of the oocytes at mid-oogenesis stage (arrows).
- First GPCR found might be involved in oocyte polarity.



Oo: oocyte; Fc: Follicle cell; TC: Trophocytes; F: follicle

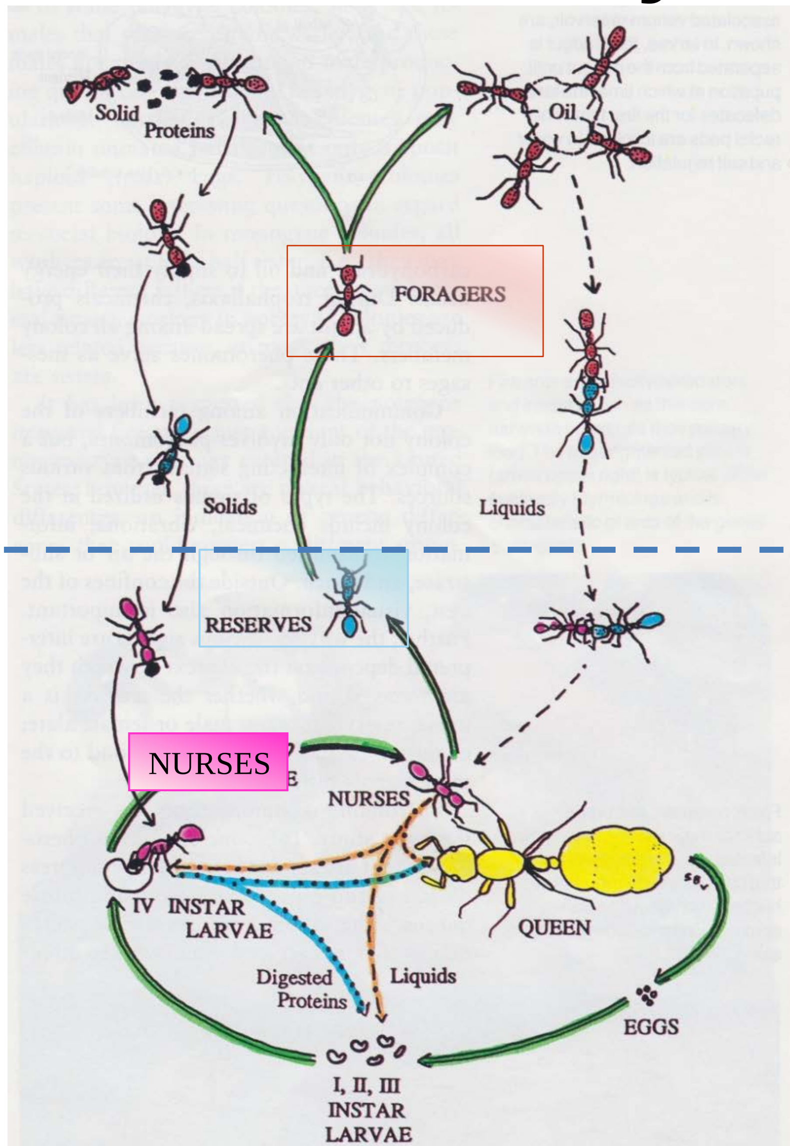
Lu and Pietrantonio *BMC Neuroscience* 2011 **12**:57

Our working model for endocrine control of reproduction in fire ant



Schematic by Dr. Pietrantonio

Nutritional status of fire ant is maintained by worker subcastes



Role of sNPF signaling in division of labor?

Vinson SB. 1997. 43(1):23-39.

First demonstration of differential expression of sNPF receptor protein in the brain of workers of social insect

OPEN ACCESS Freely available online

PLOS | ONE

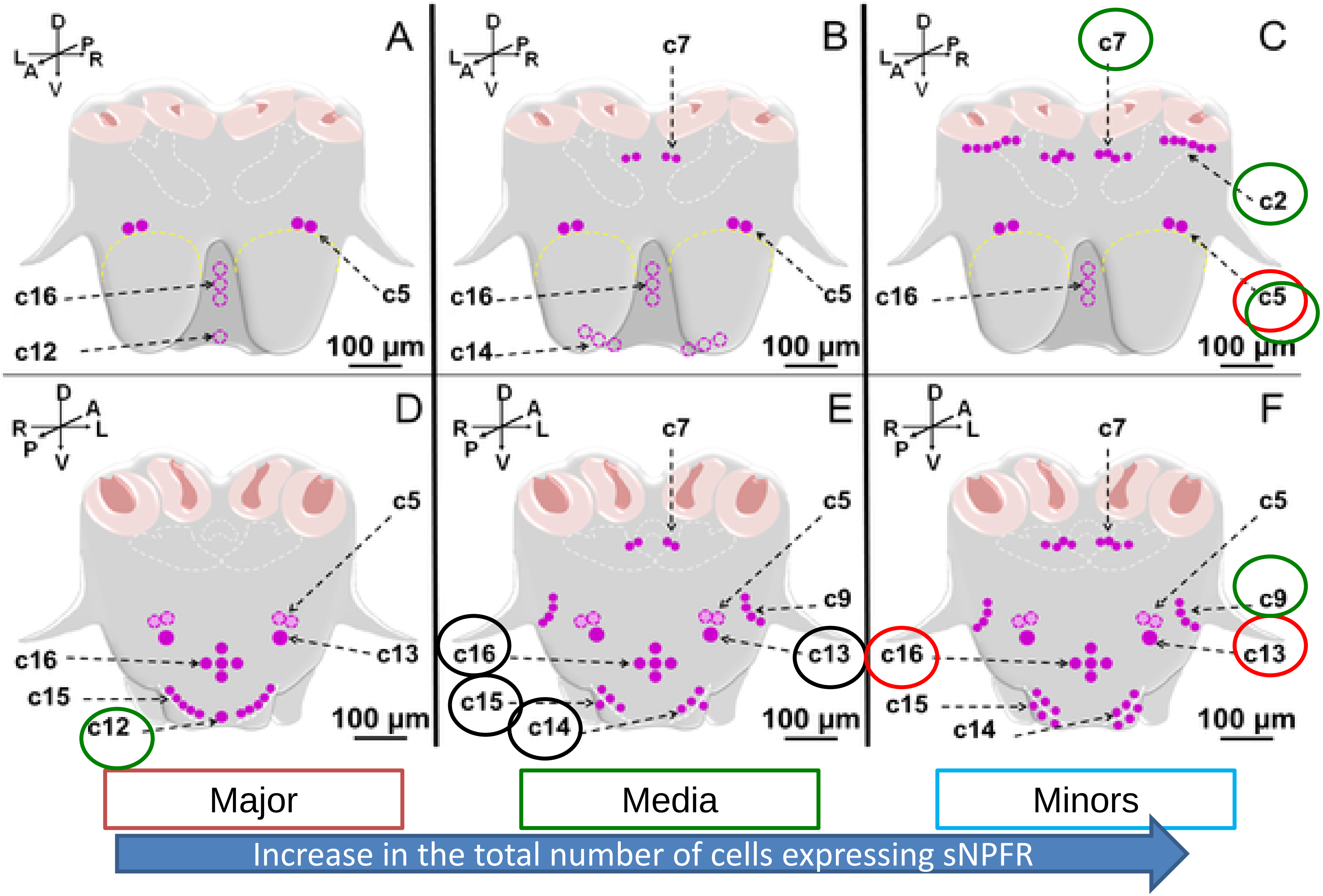
Differences in sNPF Receptor-Expressing Neurons in Brains of Fire Ant (*Solenopsis invicta* Buren) Worker Subcastes: Indicators for Division of Labor and Nutritional Status?

Paula Castillo, Patricia V. Pietrantonio*

Department of Entomology, Texas A&M University, College Station, Texas, United States of America

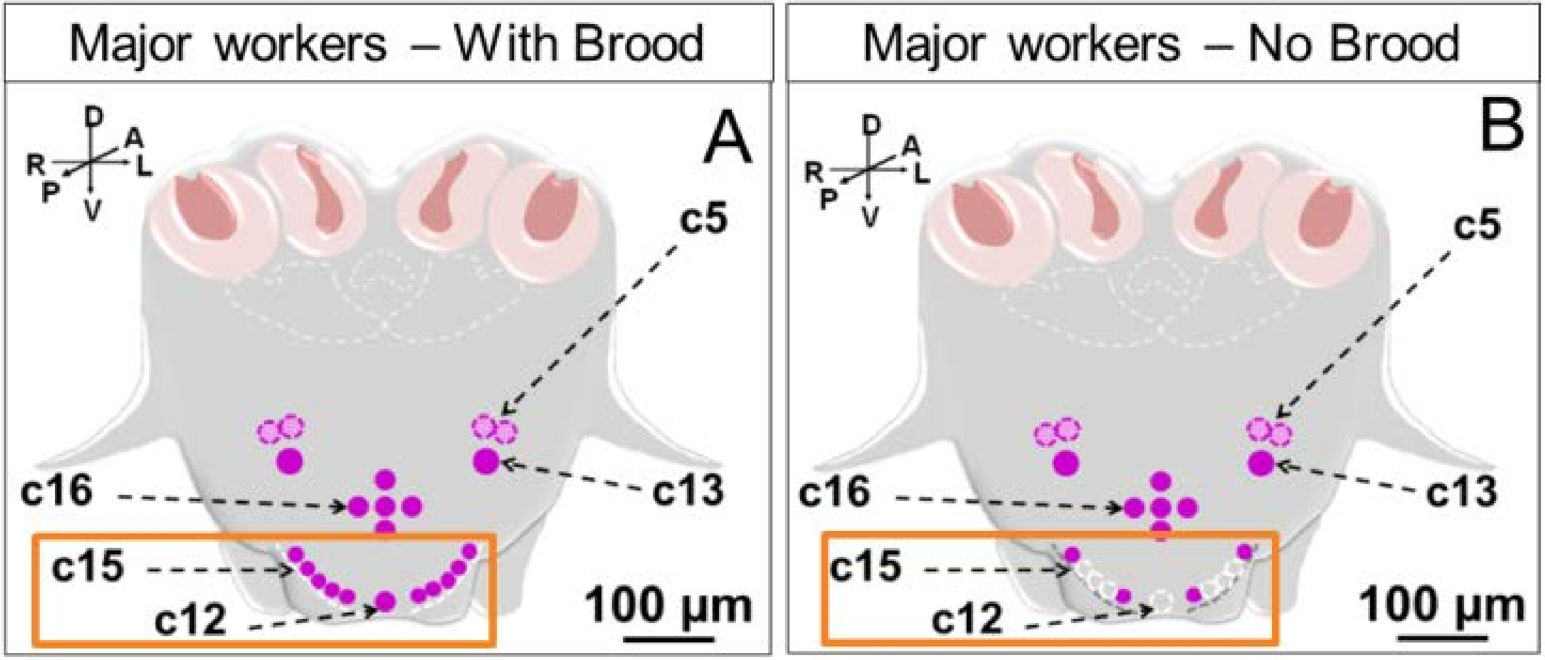
number of sNPFR cells found in minor than in major workers. Those sNPFR cells detected in all worker subcastes appear to be involved in olfaction or SEG functions. The differential expression of clusters in subcastes suggests that sNPFR signaling is involved in regulating behaviors associated with specific subcastes and thus, division of labor. Some sNPFR cells appear to be involved in nutrient sensing and/or brood care, feeding behavior and locomotion. In colonies without brood, workers showed a lower cluster number, and an overall reduced sNPFR signal. Our results suggest the sNPF signaling system is a candidate for the neurobiological control of worker division of labor and sensing brood presence, perhaps correlating with protein requirements and availability.

sNPFR in the brain of worker subcastes



Castillo P, Pietrantonio PV (2013) PLoS ONE 8: e83966.

Nutritional need of the colony changes sNPFR expressing cells



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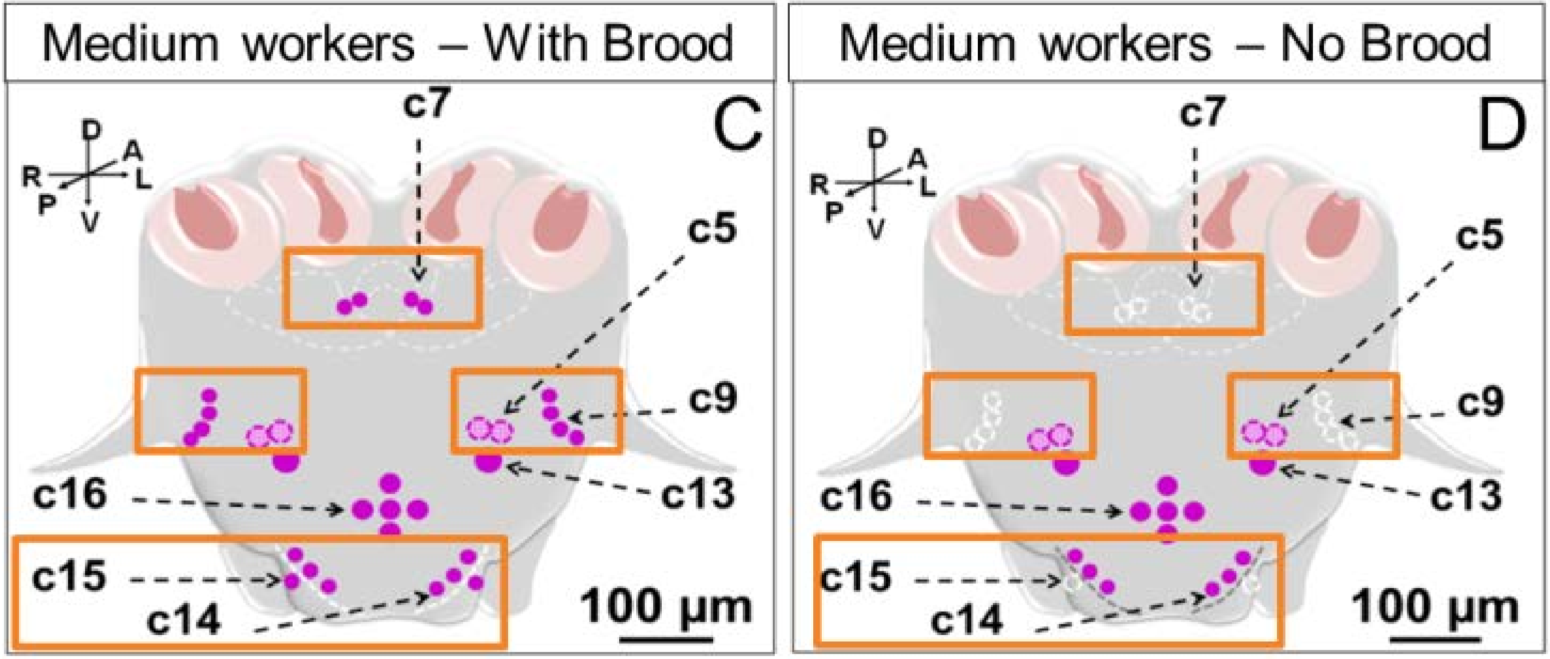

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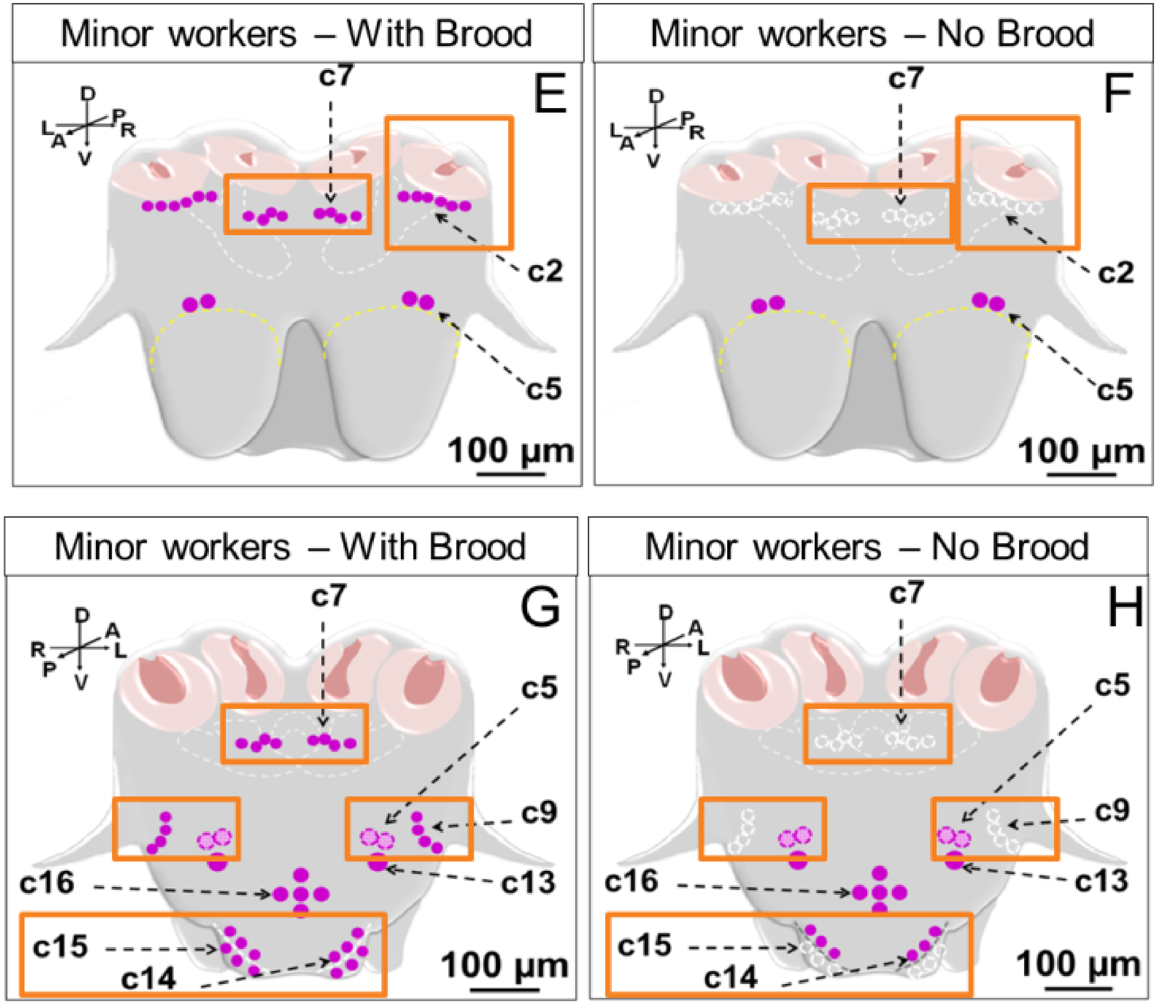


Castillo P, Pietrantonio PV (2013) PLoS ONE 8: e83966.

The type and number of immunoreactive sNPFR cell changes in worker brain

	Workers from colonies with brood						Workers from colonies without brood						Queens from colonies [2]	
	Majors		Mediums		Minors		Majors		Mediums		Minors			
Cluster	Cell Nº per brain/SEG hemisphere [1]	Total Nº of cells per brain	Cell Nº per brain/SEG hemisphere [1]	Total Nº of cells per brain	Cell Nº per brain/SEG hemisphere [1]	Total Nº of cells per brain	Cell Nº per brain/SEG hemisphere [1]	Total Nº of cells per brain	Cell Nº per brain/SEG hemisphere [1]	Total Nº of cells per brain	Cell Nº per brain/SEG hemisphere [1]	Total Nº of cells per brain	Cell Nº per brain/SEG hemisphere [1]	Total Nº of cells per brain
C1	-	-	-	-	-	-	-	-	-	-	-	-	N/A	3
C2*	-	-	-	-	4- 10	8- 20	-	-	-	-	-	-	25	50
C3	-	-	-	-	-	-	-	-	-	-	-	-	8	16
C4	-	-	-	-	-	-	-	-	-	-	-	-	6	12
C5	2- 3	4- 6	2	4	2	4	2	4	2	4	2	4	3	6
C6	-	-	-	-	-	-	-	-	-	-	-	-	N/A	30
C7*	-	-	2- 4	4- 8	4	8	-	-	-	-	-	-	4	8
C8	-	-	-	-	-	-	-	-	-	-	-	-	11	22
C9*	-	-	4	8	4	8	-	-	-	-	-	-	4	8
C10	-	-	-	-	-	-	-	-	-	-	-	-	1	2
C11	-	-	-	-	-	-	-	-	-	-	-	-	1	2
C12*	N/A	2- 3	-	-	-	-	-	-	-	-	-	-	N/A	5
c13	1	2	1	2	1	2	1	2	1	2	1	2	-	-
c14	-	-	2 - 3	4- 6	3	6	-	-	2- 3	4- 6	3	6	-	-
c15*	3- 5	6- 10	1 - 3	2- 6	3	6	1- 2	2- 4	-	-	-	-	-	-
c16	N/A	5	N/A	5	N/A	5	N/A	5	N/A	5	N/A	5	-	-
Total cell number (range)		19-26		29-39		47-59		13-15		15-17		17		164
Percent change in cell Nº [3]								32-42		48-56		63-71		

- Short neuropeptide F signal may play important role in the fire ant subcaste division of labor.

- The change in the expression of sNPF receptor in the fire ant workers’ brain in presence/absence of brood may be related to the nutritional need of the colony.

What activates the receptor?

Grand challenge in deorphanizing the short neuropeptide F receptor

- Pre-genome era
- sNPF ligand not known from the fire ant

Invertebrate sNPF sequences are conserved

- short neuropeptide F (sNPF) peptides are universally characterized by a **LRLRFamide** C-terminus

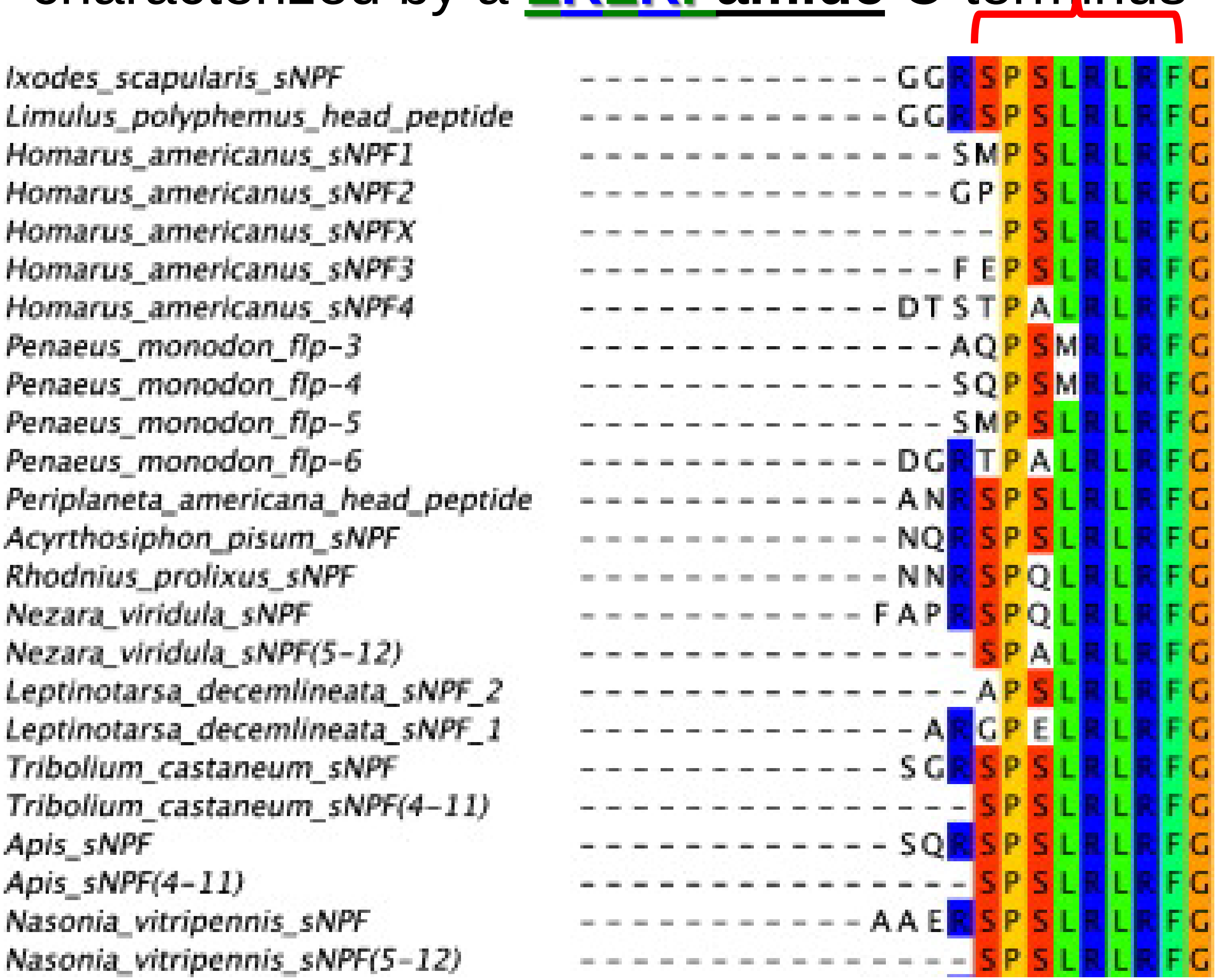
Tick
Horseshoe crab
Lobster

Shrimp

Roaches
Aphid
Chinch bug

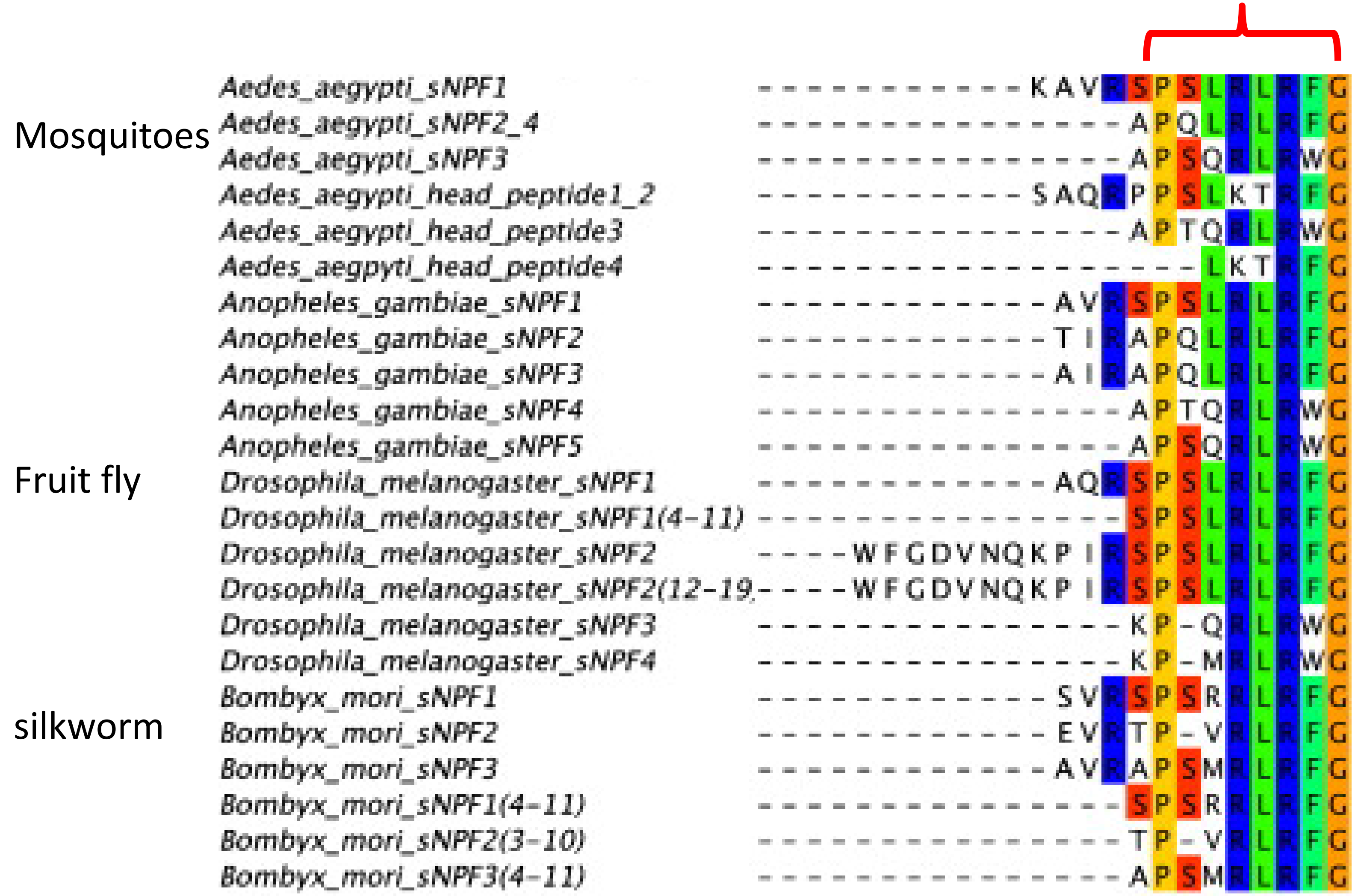
Beetles

Honey bee
Wasp



Nässel and Wegener. 2011. Peptides. 32: 1335-1355

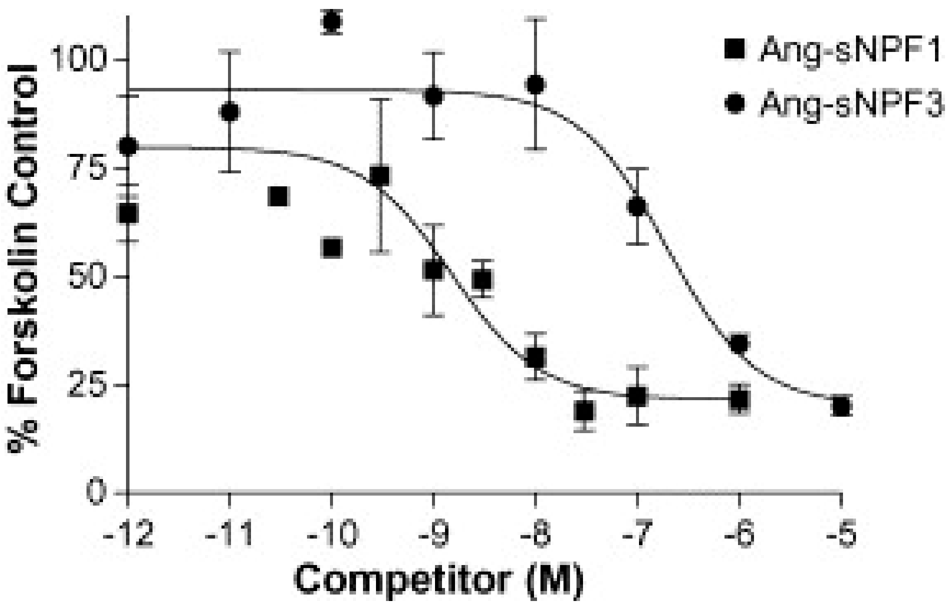
Alignment of invertebrate sNPF sequences



Nässel and Wegener. 2011. Peptides. 32: 1335-1355

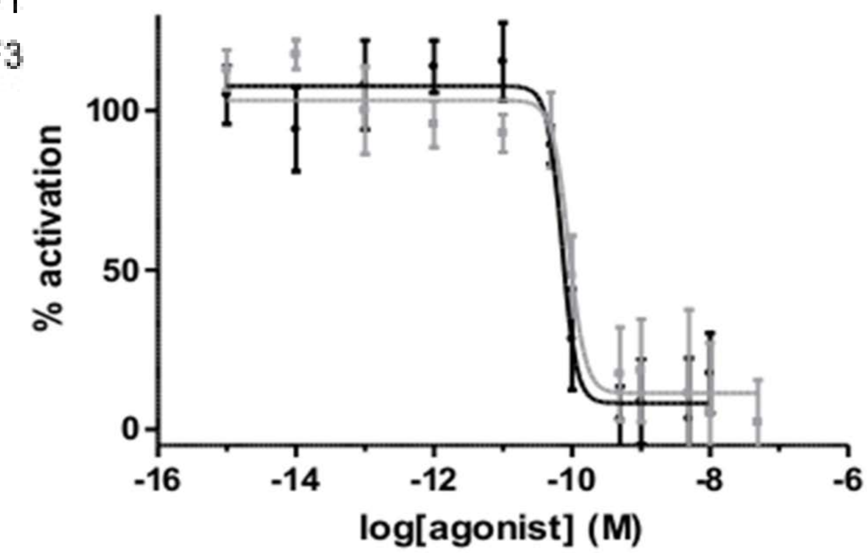
Hypothesis: sNPF receptor is Gi-protein coupled receptor

<u>Mosquito</u>



Garczynski et al. 2007.Peptides. 28,: 109 - 118

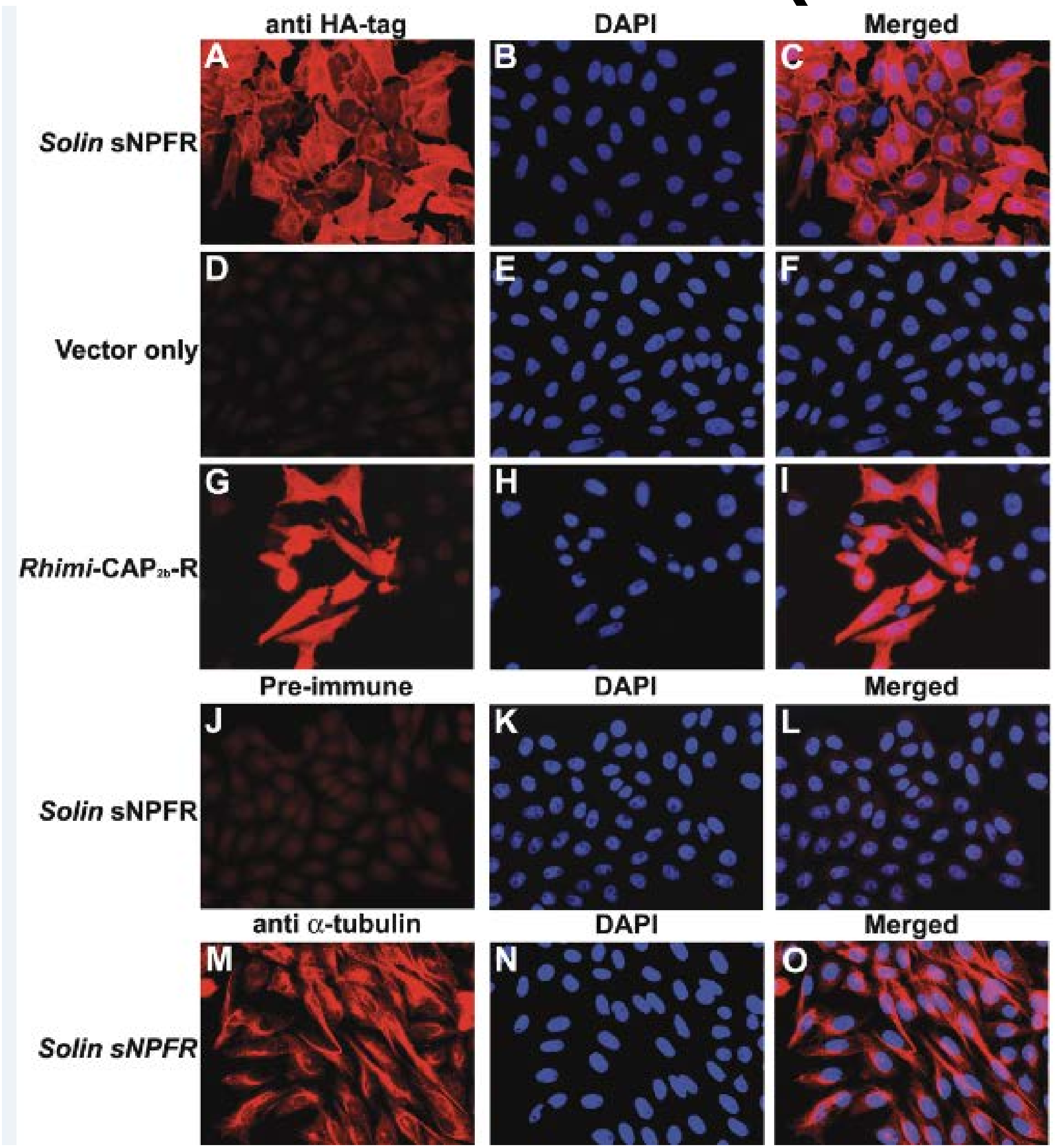
<u>Locust</u>

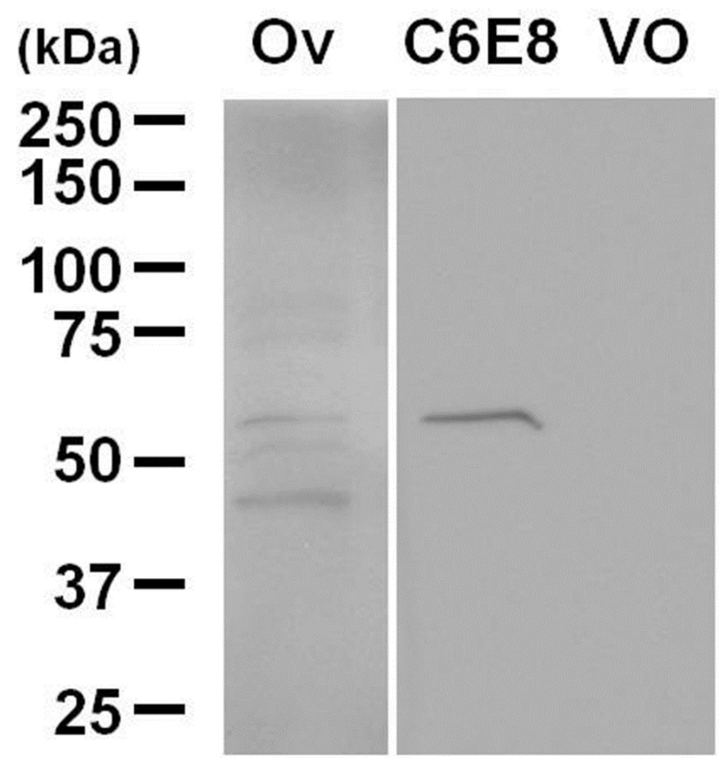


Dillen et al. 2013. PLoS ONE 8: e53604

- Gαi inhibits adenylyl cyclase decreasing intracellular cyclic adenosine monophosphate (cAMP)

Expression of fire ant sNPFR in mammalian cell line (CHOK-1 cells)





Bajracharya et al. (2014) PLoS ONE

Ligands tested on the *Sis*NPFR-C6E8 cell line CHO-K1 stably expressing the sNPF receptor

(Hsiao-Ling Lu's Ph.D. work)

Peptide ligands	Amino acid sequence (a=amidated at C-terminus)	Activity (EC_{50})
*Drome*sNPF1	AQRSPSLRLRFa	not active 1µM
*Drome*sNPF2	WFGDVNQKPIRSPSLRLRFa	not active 1µM
Drome sNPF2 12-19	SPSLRLRFa*	not active 1µM

Pre-OMICs era offered no solution

Draft genomes of six species of ants were released

Fire ant genome (2010)

Home

Genome Browsers

Genome Browsers are available for the following species:

1. Argentine Ant, *Linepithema humile*
2. Carpenter Ant, *Camponotus floridanus*
3. Fire Ant, *Solenopsis invicta*
4. Jumping Ant, *Harpegnathos saltator*
5. Leaf Cutter Ant, *Atta cephalotes*
6. Red Harvester Ant, *Pogonomyrmex barbatus*

Using the honey bee short neuropeptide F sequence we identified the sNPF gene in the fire ant genome, but…………

http://hymenopteragenome.org

The endogenous "sNPF propeptide" reveals a <u>sNPY</u> ligand!

cDNA cloning and predicted amino acid sequence

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                                                                                   ACATGGGGACACTTCGCATCATTACCGCCGTAA     -88
CGTGCCAGCCACTCAATCCCCGTGCCGCTCGTCTCTCATCACCTGAGACTAACGCTCAAGAATTCGAACCCGAGGACAGAGGAGGCC      -1
ATG TAT GCT AAA CGT TGC GCA GCT TTC GTT CTT TTC GTC GTG ATA GTC GTG ATA GTC GGC CTC GTG      66
 M   Y   A   K   R   C   A   A   F   V   L   F   V   V   I   V   V   I   V   G   L   V
GAC GCC ACT GAA AAT TAC GTG GAC TAC GGA GAA GAA ATG GCG GAG AAA GCG CCC GCG GAG AAC ATC     132
 D   A   T   E   N   Y   V   D   Y   G   E   E   M   A   E   K   A   P   A   E   N   I
CAC GAA TTG TAC AGA CTC TTG TTG CAA CGC AAT ACC TTG GAT AAT GGC TTT GGC GAC ATC CCC CCG     198
 H   E   L   Y   R   L   L   L   Q   R   N   T   L   D   N   G   F   G   D   I   P   P
CAG CAA TTG ATA CTC CGG AAG TCG CTG CGA TCG GCT TTA GCT GCG GGA CAT CTT CGG TAC GGT CGT     264
 Q   Q   L   I   L   R   K   S   L   R   S   A   L   A   A   G   H   L   R   Y   G   R
TCT GGA TCA CAA TTT TCC GCG AGA GCT CTG TCG AGA CCT CTG GCT GTA GCG GGA CGA TAC GAC GAC     330
 S   G   S   Q   F   S   A   R   A   L   S   R   P   L   A   V   A   G   R   Y   D   D
AAC AAT TAA TCGGTGAGGCTGTTCCAGGGACACCTTTCCGATTTATTTCTTGATAAAAAACGCGCGACTACCCTCTTTACAATA     414
 N   N   .
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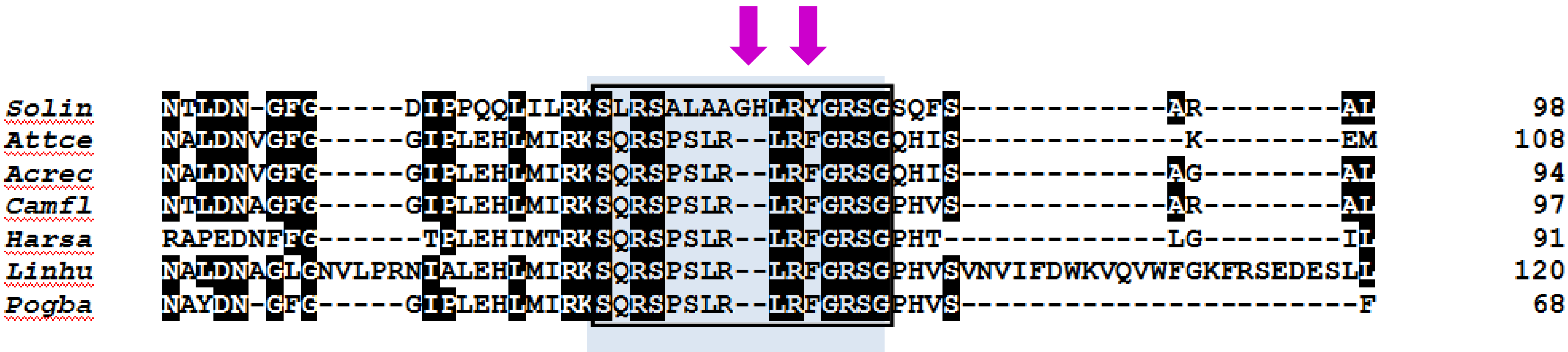
Different from the universally conserved LRLRFamide

Bajracharya et al. (2014) PLoS ONE. 9: e109590

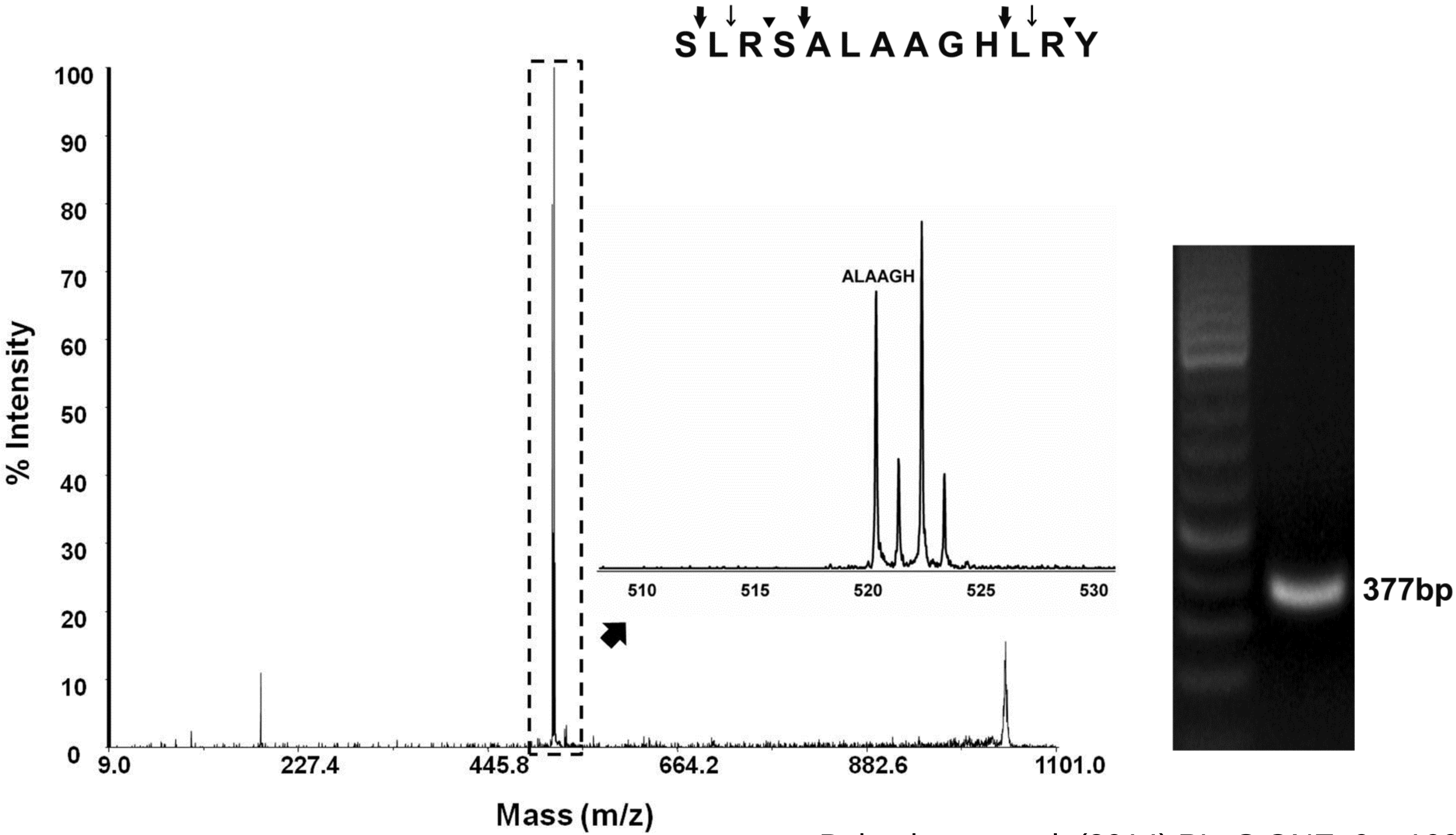
The fire ant predicted active “sNPF” ligand is different from that of other invertebrates and different from the other ant species



Bajracharya et al. (2014) PLoS ONE. 9: e109590

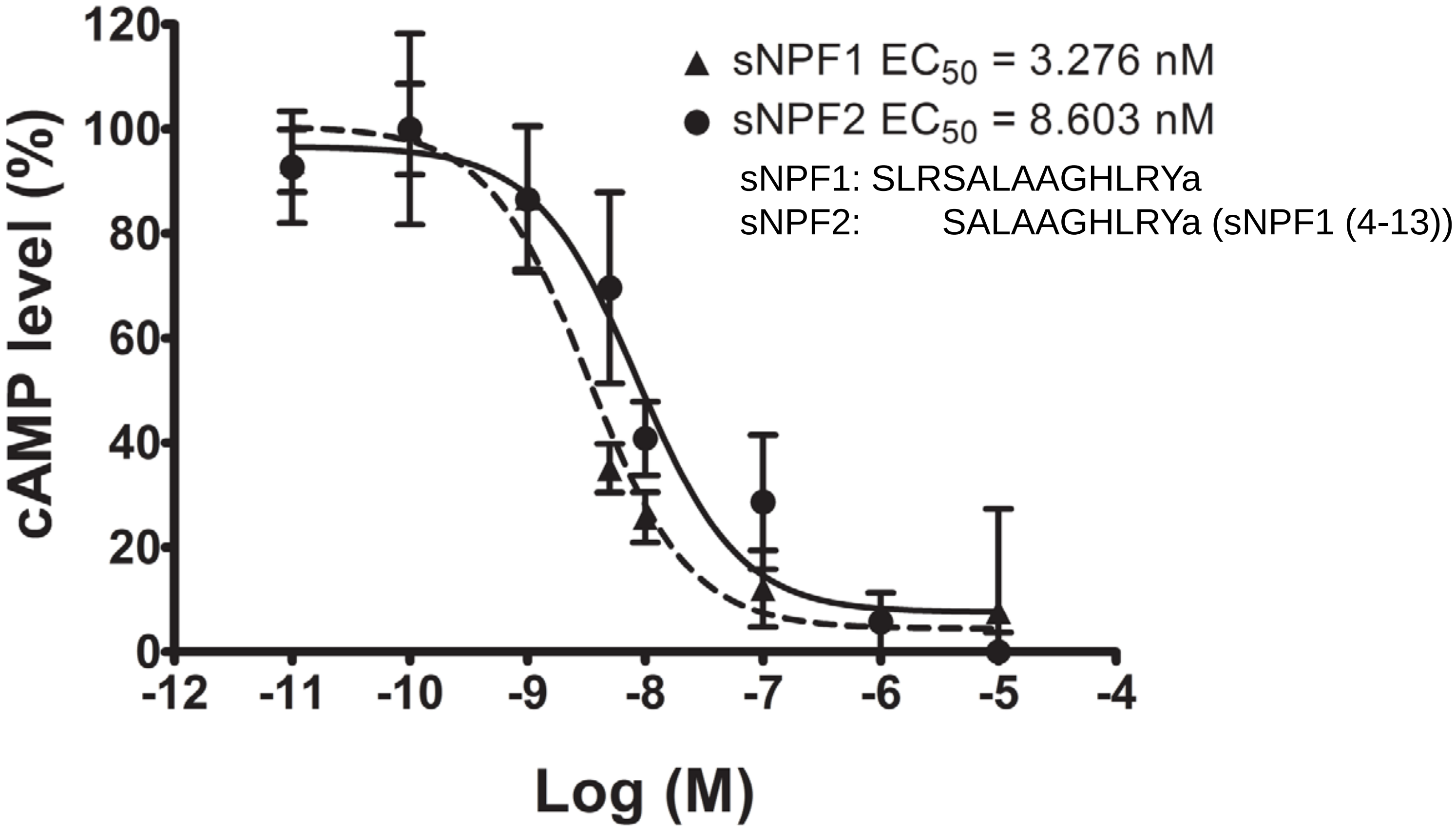
The predicted sNPY peptide is expressed in fire ants

Identification of sNPY from the fire ant eggs and larvae in MALDI-TOF MS



Bajracharya et al. (2014) PLoS ONE. 9: e109590

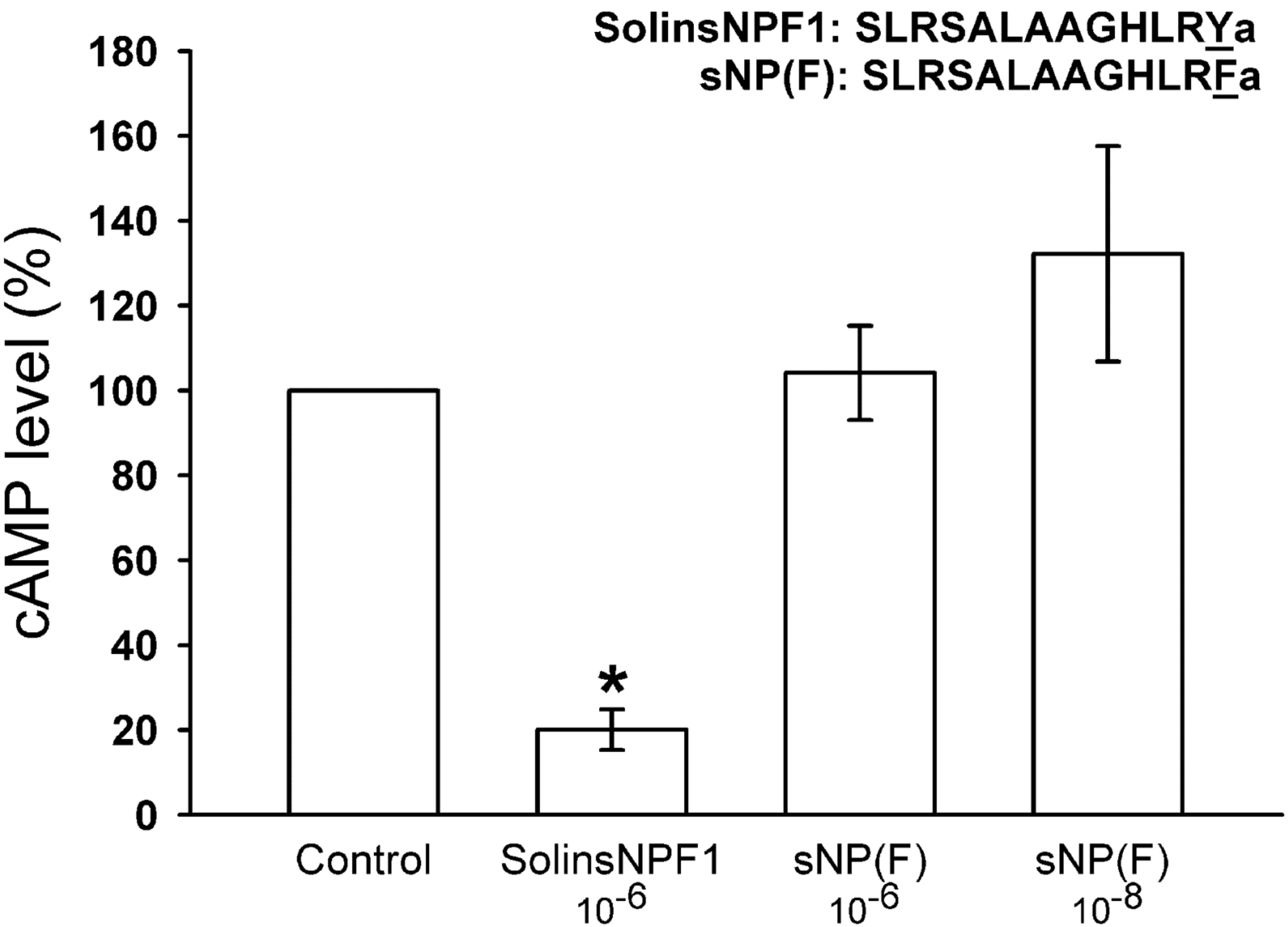
The predicted peptide activates the fire ant "sNPF" receptor



Endogenous sNPY decreases intracellular cAMP in CHO-K1 cells

Bajracharya et al. (2014) PLoS ONE. 9: e109590

Tyrosine (Y) at the peptide C-terminus is critical for receptor-ligand binding: Phe (F) renders fire ant peptide inactive



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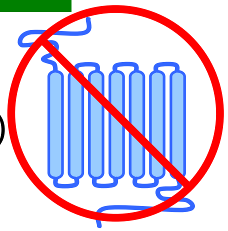
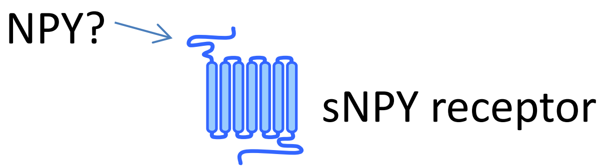
What about NPF peptides/receptors in fire ants?



- The fire ant genome lacks the insect long NPF receptor!
- The honey bee genome also lacks the long NPF receptor (Ament et al. 2011))

Do other NPY ligands perhaps activate the sNPY receptor?



Ligands tested on the fire ant sNPF receptor (*Si*sNPFR-C6E8 cell line)

Peptide ligands	**Amino acid sequence (a= amidated at C-terminus)**	**Activity (EC_{50})**
Mouse PYY	**AKPEAPGEDASPEELSRYYASLRHYLNLVTRQRYa**	**not active 1μM**
***Apime* NPY**	**EPEPMARPTRPEIFTSPEELRRYIDHVSDYYLLSGKARYa**	**not active 1μM**

- **Honey bees though, encode the long NPF peptide which ends in NPY**
- **It is unknown if fire ants encode a canonical long NPF peptide.**

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Conclusions

- Endogenous sNPY ligand was identified in the fire ant
- Fire ant sNPY ligand is different from the consensus sequence xPxLRLRFamide universal among invertebrates characterized so far
- sNPF receptor is a Gi-coupled protein receptor because it decreases cAMP

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The Red Imported Fire Ant (*Solenopsis invicta* Buren) Kept Y not F: Predicted sNPY Endogenous Ligands Deorphanize the Short NPF (sNPF) Receptor

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Abstract

Neuropeptides and their receptors play vital roles in controlling the physiology and behavior of animals. Short neuropeptide F (sNPF) signaling regulates several physiological processes in insects such as feeding, locomotion, circadian rhythm and reproduction, among others. Previously, the red imported fire ant (*Solenopsis invicta*) sNPF receptor (*S. invicta* sNPFR), a G protein-coupled receptor, was immunolocalized in queen and worker brain and queen ovaries. Differential distribution patterns of *S. invicta* sNPFR protein in fire ant worker brain were associated both with worker subcastes and with presence or absence of brood in the colony. However, the cognate ligand for this sNPFR has not been characterized and attempts to deorphanize the receptor with sNPF peptides from other insect species which ended in the canonical sequence LRLRFamide, failed. Receptor deorphanization is an important step to understand the neuropeptide receptor downstream signaling cascade. We cloned the full length cDNA of the putative *S. invicta* sNPF prepropeptide and identified the putative "sNPF" ligand within its sequence. The peptide ends with an amidated Tyr residue whereas in other insect species sNPFs have an amidated Phe or Trp residue at the C-terminus. We stably expressed the HA-tagged *S. invicta* sNPFR in CHO-K1 cells. Two *S. invicta* sNPFs differing at their N-terminus were synthesized that equally activated the sNPFR, SLRSALAAGHLRYa (EC_{50} = 3.2 nM) and SALAAGHLRYa (EC_{50} = 8.6 nM). Both peptides decreased the intracellular cAMP concentration, indicating signaling through the $G_{\alpha i}$-subunit. The receptor was not activated by sNPF peptides from other insect species, honey bee long NPF (NPY) or mammalian PYY. Further, a synthesized peptide otherwise identical to the fire ant sequence but in which the C-terminal amidated amino acid residue 'Y' was switched to 'F', failed to activate the sNPFR. This discovery will now allow us to investigate the function of sNPY and its cognate receptor in fire ant biology.

Future directions

1. Identify the long NPY/F from the fire ant.

2. Localize the sNPY producing cells in the fire ant queen and workers brain by *in situ* hybridization.

3. Understand the physiological role of sNPY and sNPFR in the fire ant (RNAi).

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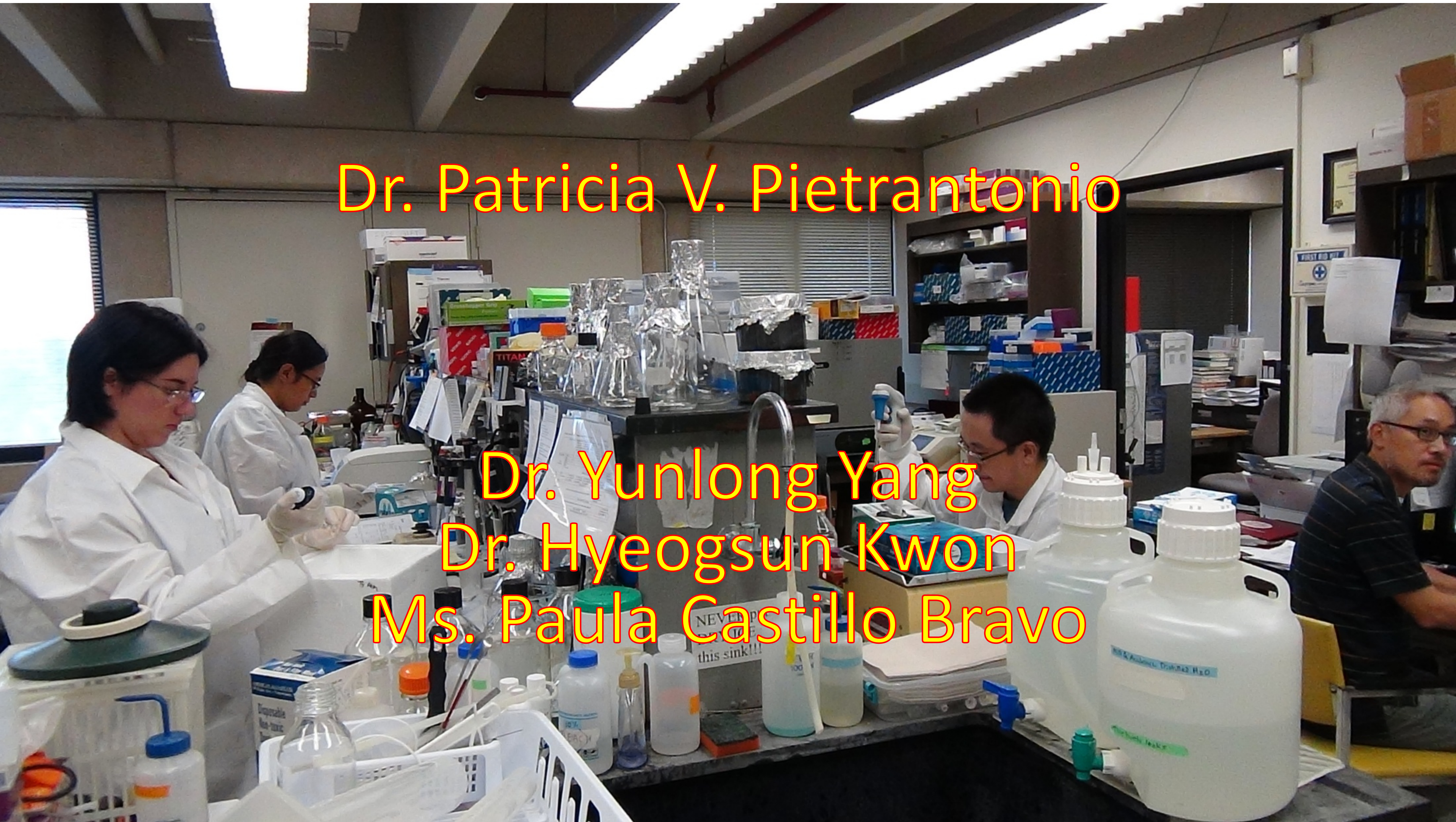
Questions?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