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.

INTRODUCTION

Fire ants are eusocial insects; they exhibit reproductive division of labor. Their colonies are composed of reproductives (queens and drones) and sterile female workers. Workers co-operate and care for the queen and brood (egg, larvae and pupae), forage for food, defend the nest, dig soil for nest construction, etc. Workers are divided loosely in subcastes (major, medium and minors) by their size and somewhat by task performed. However, the neuronal and molecular mechanisms related to this worker division of labor are poorly understood.

In insects, short neuropeptides F (sNPFs) are small peptides which exert their action through the short neuropeptide F receptor (sNPFR), a G protein-coupled receptor (GPCR) related to the mammalian NPY receptor (NPY2). sNPFs are involved in the regulation of critical functions such as feeding and growth, stress responses, locomotion, olfaction, hormone release, reproduction, and learning and memory.

Here we focused on the immunolocalization of the short neuropeptide F receptor (sNPFR) in the brain of fire ant workers subcastes. This study aims at investigating if the sNPF receptor spatial expression is associated with worker division of labor (subcastes). Experiments were also designed to determine if the sNPF receptor spatial expression changes with colony nutritional status or requirements affected by the presence or absence of brood [1].

MATERIALS AND METHODS

Insects. Polygyne colonies collected in Burleson County, TX, were maintained in the laboratory as described [1]. Colonies with and without brood (egg larvae and pupae) were used, and all had mated queens.

Classification and selection of worker ant subcastes. Ants were collected and classified into majors, mediums and minors according to the head width (Fig. 1). Workers were chosen while performing different tasks as shown in Fig. 2.

Immunofluorescence analysis of brains/SEG of worker ants. Cells expressing the sNPFR were detected by immunofluorescence in brain whole mounts with a validated antibody, as described [2]. Cell clusters were numbered following nomenclature used for the fire ant queen brain [2] and novel worker specific clusters were designated c13-c16.

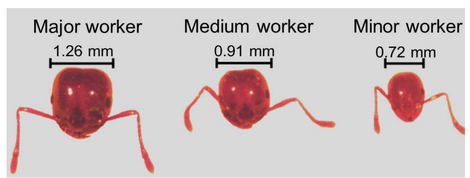
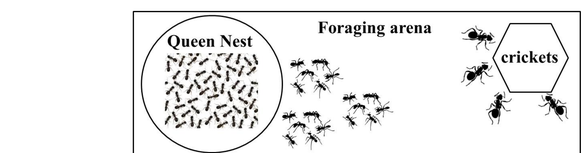


Figure 1. *Comparison of the head widths (H.W.) among fire ant worker subcastes.*



Worker subcaste	Minor	Medium	Major
Task performed	Nursing	Not specific	Foraging
Location	Inside the nest	In/Out, or around the nest	Far from the nest

Figure 2. *Diagram illustrating the nest location and task performed by worker subcastes. For this study major workers were collected while foraging for protein (crickets), far from the queen nest. Medium workers were collected inside the nest or outside, nearby. Minor workers were collected inside the nest while nursing brood. Colonies were also provided water and honey-water ad libitum (not shown).*

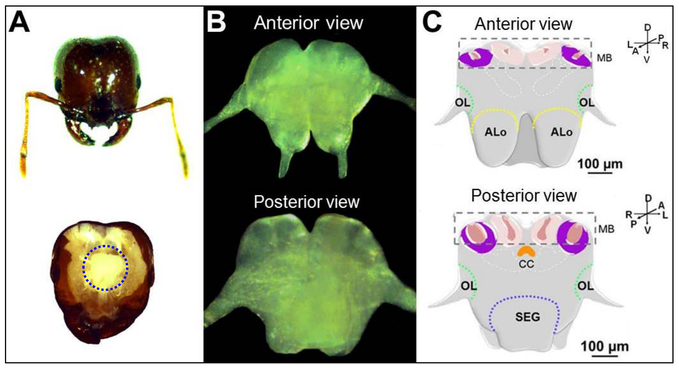


Figure 3. *Structure of the head and brain with subesophageal ganglion of worker ants. (A) the head of a worker (top) and brain inside the head capsule (bottom, inside dashed circle). (B) The ant brain is shown in two views. (C) A schematic of brain structures as follows: MB = mushroom bodies (in grey dashed rectangles. medial calyces in pink, lateral calyces in purple); OL = optic lobe; ALo = antennal lobe; CC = central complex (in orange); SEG = subesophageal ganglion.*

RESULTS

Different cell clusters could be observed only from either the anterior or posterior view of the brain (Fig. 3) and others from both. A total maximum of 9 cell clusters expressing the sNPFR are present across worker subcastes: c2, c5, c7, c9, c12 and c13-16 (Fig. 4). A schematic summarizing the location of the cell clusters expressing the sNPFR is shown in Fig. 4. Some of the sNPFR cell clusters were highly reminiscent of those observed in the queen brain (Table 1). Novel clusters found exclusively in workers were numbered c13-c16 (Fig. 4; Table 1). The total number of cells expressing the sNPF receptor decreases from minor to major workers. Minor worker ants exhibited a total of eight cell clusters (total of 47–59 sNPFR cells), while major workers showed five clusters (19–26 sNPFR cells). Medium workers were intermediate in immunolabeled cell number, having seven clusters of cells (29–39 sNPFR cells) (Table 1). In colonies without brood clusters c5, c13, c14 and c16 remained unchanged with respect to colonies with brood, but in other clusters the number of sNPFR immunoreactive cells was considerably reduced in comparison to workers of colonies with brood (Fig. 5). Fluorescent photography of a minor worker brain clearly showing sNPFR immunolabeled clusters is shown in Fig. 6.

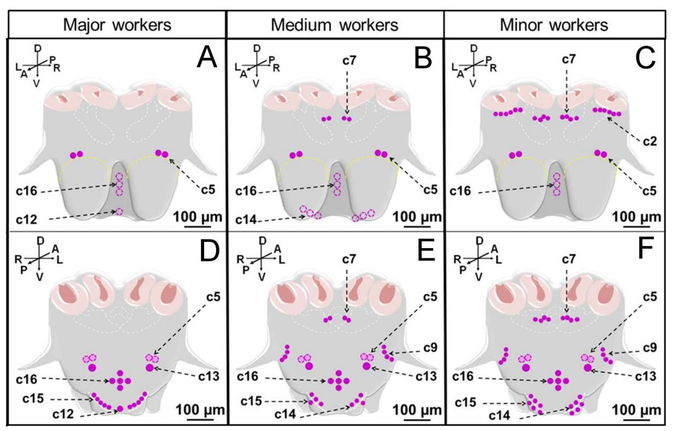


Figure 4. *Schematic of the sNPFR immunolocalization in the brain and SEG of worker subcastes in colonies with brood. Anterior (top panel) and posterior (bottom panel) views of the brain show different cell clusters expressing the sNPFR (purple dots). (A, D) show the immunolocalization of the sNPFR in majors; (B, E) in mediums and (C, F) in minors. Dashed-empty circles indicate cells that can be seen faintly from the anterior view; purple checkered-filled circles indicate the same, but when the brain is seen from the posterior side.*

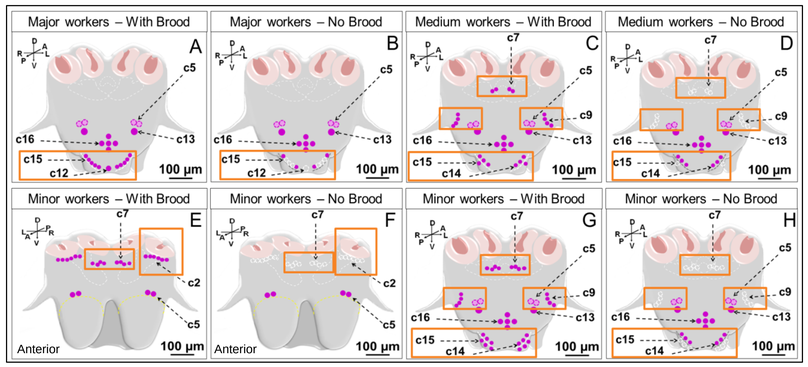


Figure 5. *Summary schematic of brain and subesophageal ganglion immunolocalization of the sNPFR in worker subcastes from colonies without brood, and side by side comparisons with those with brood. Areas in orange boxes enclose specific brain areas for comparison. Dashed white circles correspond to the expected location of immunoreactive cells present in the brain of workers from colonies with brood that are not immunolabeled in colonies without brood. A–D, G and H show the receptor signal in the posterior brain; E and F show the anterior brain view. Note the overall decrease in the number of cells immunostained in worker brains from colonies without brood.*

Table 1. Number of sNPFR immunoreactive cells detected in the brain of worker subcastes from colonies with and without brood and comparison to those reported previously for queens.

	Workers from colonies with brood						Workers from colonies without brood						Queens from colonies (2)	
	Majors		Mediums		Minors		Majors		Mediums		Minors			
Cluster	Cell N° per brain/SEG hemisphere(1)	Total N° of cells per brain	Cell N° per brain/SEG hemisphere(1)	Total N° of cells per brain	Cell N° per brain/SEG hemisphere(1)	Total N° of cells per brain	Cell N° per brain/SEG hemisphere(1)	Total N° of cells per brain	Cell N° per brain/SEG hemisphere(1)	Total N° of cells per brain	Cell N° per brain/SEG hemisphere(1)	Total N° of cells per brain	Cell N° per brain/SEG hemisphere(1)	Total N° 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 N° (3)								32-42		48-56		63-71		

Footnotes: Note that clusters only present in the midline of the brain, and therefore not symmetrically distributed are: C1, C6, C12 and c16; clusters c13 through c16 are exclusively found in workers, but c14 is absent in majors. (1)The number of cells per cluster in one brain/SEG hemisphere is indicated only for clusters that show a symmetrical distribution; in workers, numbers separated by a hyphen indicate the range in the number of cells observed in different individuals. N/A refers to clusters in the midline of the brain which cell number is only indicated in the total number per brain column. (2) From Lu et al [2]. (3) The percentage decrease in the cell number range per subcastes in colonies without brood was calculated with respect to the respective range in cell numbers in colonies with brood. Clusters in pink are queen exclusive; in green, queen and majors exclusive; in light blue common to mediums, minors and queens; in white, minors and queen exclusive. In yellow are worker clusters that are present regardless of presence or absence of brood; in light yellow is a cluster common to all females (all worker subcastes and queen). In workers, clusters with asterisks change in cell number depending on the presence or absence of brood; c15 is the only worker exclusive cluster that responds to the absence of brood (in orange).

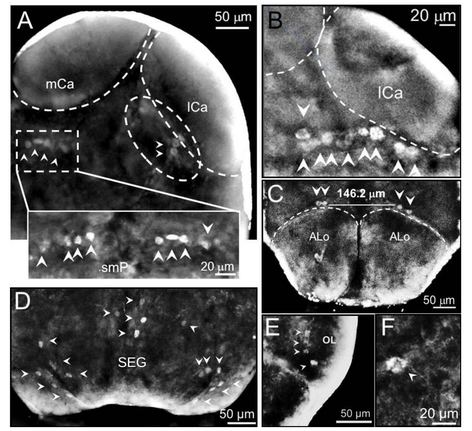


Figure 6. *Clusters of sNPF receptor expressing cells in a minor worker. (A) cluster c7 (dashed rectangle; detail in inset) and c2 (dashed oval) are located near the mushroom bodies. (B) c2 is composed of several cells. (C) c5 is located near the antennal lobes. (D) clusters c14-c16 are located in the SEG. (E) c9 is located close to the optic lobes. (F) detail of c13 in the posterior brain view.*

DISCUSSION

The spatial expression pattern of the sNPFR in the fire ant is reported for the first time in workers of a social insect. Some of the cell clusters expressing the sNPF receptor in workers were observed before in the queen brain, suggesting a possible role of the sNPF receptor in the regulation of both, common and independent neuronal circuits in queens and workers, which could be involved in mechanisms of nutrient sensing, brood care, locomotion, vision and feeding behaviors.

The spatial expression of sNPFR is different among worker subcastes. Additionally, the number of clusters and immunoreactive cells within clusters are considerably reduced in the brain of workers from colonies without brood. The brood plays an important role in the nutrition of the colony because 4th instar larvae digest large amounts of protein, which is shared with others members of the colony via trophallaxis. In brood-less colonies, the availability of protein is diminished, as there is no colony growth, thus the worker brain appears to reflect this through a reduced expression of the sNPF receptor in all subcastes.

CONCLUSIONS

- The sNPF receptor spatial expression is different in brains of workers of different subcastes, maybe due to its involvement in the regulation of specific tasks associated with division of labor.
- The sNPF receptor spatial expression is sensitive to changes in the nutritional status of the colony. When the protein availability decreases, the expression of this receptor is considerably reduced, suggesting it could be involved in the regulation of nutritional sensing mechanisms, which are related to the modulation of foraging behavior.

REFERENCES

1 Castillo P, Pietrantonio PV. (2013) Differences in sNPF receptor-expressing neurons in brains of fire ant (*Solenopsis invicta* Buren) worker subcastes: indicators for division of labor and nutritional status?. PLoS ONE 8(12): e83966.

2 Lu H, Pietrantonio PV. (2011) Immunolocalization of the short neuropeptide F receptor in queen brains and ovaries of the red imported fire ant (*Solenopsis invicta* Buren). BMC Neurosci 12: 57.