

projecting. Four eyes in two diads. Ratio of eyes 2.0. Posterior lateral eyes separated by 3 times their diameter, lateral eyes of each side contiguous. Legs covered with setae and bristles, no spines. Leg formula 1243. Leg measurements: I: femur 0.30/patella 0.14/tibia 0.22/metatarsus 0.14/tarsus 0.24/total 1.04. II: 0.28/0.12/0.20/0.12/0.22/0.94. III: 0.22/0.12/0.12/0.12/0.20/0.78. IV: 0.26/0.12/0.18/0.14/0.22/0.92. Palpal sperm duct with anterior sinuosity, median tegular apophysis inconspicuous (Fig. 22).

*Female*: Unknown.

*Variation*: Three males: total length 0.50–0.54; carapace length 0.22–0.28; femur I length 0.26–0.32.

*Natural history*: All specimens were collected in a cave, approximately 200 m from the entrance, in piles of organic litter, using Winkler extractors.

*Distribution*: Central-eastern Brazil, state of Goiás.

*Other material examined*: Only the types.

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### Possible role of serotonin in the regulation of feeding in the tarantula *Aphonopelma hentzi* (Girard) (Orthognatha, Theraphosidae)

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### Summary

Exogenous administration of 5-hydroxytryptamine (5-HT, serotonin) caused a significant decrease in body weight (12.9% weight loss) and in the number of prey consumed over a 10-week feeding period, in adult females of the tarantula *Aphonopelma hentzi*, as compared with non-treated controls (NTC) and spiders injected with physiological saline (SAL). Injection of methyltryptophan (MTP), a 5-HT antagonist, caused an increase in number of prey consumed and body weight (25.7%) as compared with NTC and SAL animals. No significant differences were found between NTC and SAL spiders with respect to changes in body weight or number of prey eaten. NTC spiders fed on crickets *ad libitum* exhibited an increase in body weight of 7.8% after 10 weeks. The possible role of 5-HT in the regulation of feeding behaviour in arthropods is discussed.

### Introduction

It is well known that alterations in neurotransmitter/neuromodulator/neurohormone levels in mammals can affect the regulation of feeding and the amount of food consumed (Fernstrom & Wurtman, 1971). In addition, it has been argued that an increase in the intake of certain nutrients, such as carbohydrates, can result in an increased rate of synthesis of 5-hydroxytryptamine (5-HT, serotonin) in the blood and central nervous system (CNS) (Thibault & Booth, 1999). This increase in serotonin synthesis can then alter feeding behaviour by decreasing the animal's intake of carbohydrates.

The association between 5-HT and food consumption also occurs in insects. Cohen *et al.* (1988) demonstrated that the alteration of 5-HT levels had a significant effect on carbohydrate intake in noctuid caterpillars. As a result of a reduction in 5-HT concentrations caused by the administration of methyltryptophan, a serotonin antagonist, caterpillars increased their overall feeding rate as well as their rate of carbohydrate intake. Conversely, caterpillars ingested less food and carbohydrates when 5-HT levels were artificially increased. More recently, Cohen (2001) showed that injections of 5-HT promoted decreases in overall feeding and carbohydrate

intake of 8.3% and 9.7%, respectively, in nymphs of the cockroach *Rhyparobia maderae*. In contrast, injections of methyltryptophan caused a 2.9% increase in overall feeding and a 7.1% increase in carbohydrate intake.

To my knowledge, there are no comparable data on the possible role of 5-HT in the regulation of feeding behaviour in arachnids. The purpose of this study was to determine if there is any association between overall food intake and 5-HT in the tarantula *Aphonopelma hentzi* (Girard).

## Methods

Experiments were conducted on adult females of *A. hentzi* originally collected in Elgin, Texas in 1998 and maintained in the laboratory under conditions described previously (Punzo & Henderson, 1999). Females were used because they have significantly longer life spans and feed throughout their adult lives (Baerg, 1958; Punzo & Henderson, 1999). All spiders used in these experiments were of approximately equal size ( $7.0 \pm 0.15$  g).

Sixty female spiders were chosen randomly and divided into 4 groups, with each group containing 15 females: (a) Group 1 – spiders injected with 5-HT; (b) Group 2 – injected with methyltryptophan (antagonist); (c) Group 3 – injected with physiological saline; (d) Group 4 – non-treated controls. Injection procedures followed those previously described by Punzo (1988) for *Aphonopelma chalcodes*. To summarise, spiders that were to receive injections were first anaesthetised with CO<sub>2</sub>, and then injected with 12  $\mu$ l of either 2.7 mg/kg 5-HT, 2.7 mg/kg methyltryptophan (Sigma Chemical Co., St. Louis, Missouri), or 0.7% physiological saline, depending on their experimental group. These concentrations were selected on the basis of previous pilot

dose-response experiments (unpubl. data). Injections were made between the sternum and coxa of the second leg. Spiders were allowed to recover for a period of 24 h and then deprived of food for two weeks before testing.

Following this deprivation period, individually housed spiders from each group were weighed to the nearest 0.1 mg on a Sartorius electrical analytical balance, and provided with crickets (*Acheta domesticus*) ad lib, thus allowing them to feed to satiation. Ad lib feeding was continued for a period of 10 weeks. The number of crickets consumed by each spider as well as the spider weights were recorded after the 10 week feeding period. The crickets used were of similar sizes ( $0.4 \pm 0.02$  g) and they were dusted with a multiple vitamin, mineral and amino acid supplement powder (Formula V/M/A, Bush Supply, Neodesha, Kansas).

Statistical procedures followed the protocols described by Woolf (1968). Bartlett's test for homogeneity of variances showed equality of variances for population samples. Data on the overall weight changes after the 10-week feeding period were analysed using a completely randomised block-design (Model I, one-way analysis of variance, ANOVA) with four treatments (groups 1–4) ( $n=15$  spiders/group). Data on the number of crickets consumed/week (10 weeks) were analysed using a factorial design ANOVA (factor A, weeks of feeding; factor B, groups 1–4). Differences between individual means in both cases were tested for significance using a Student-Newman-Keuls (SNK) multiple range test.

## Results

The weights of the four groups of spiders (Groups 1–4) at the end of the 10-week feeding period are shown in Fig. 1. There was an overall significant effect of treatments on weight change ( $F=13.98$ ,  $df=3$ , 56,  $p<0.01$ ). No significant differences were found between non-treated controls (NTC) and saline-injected (SAL)

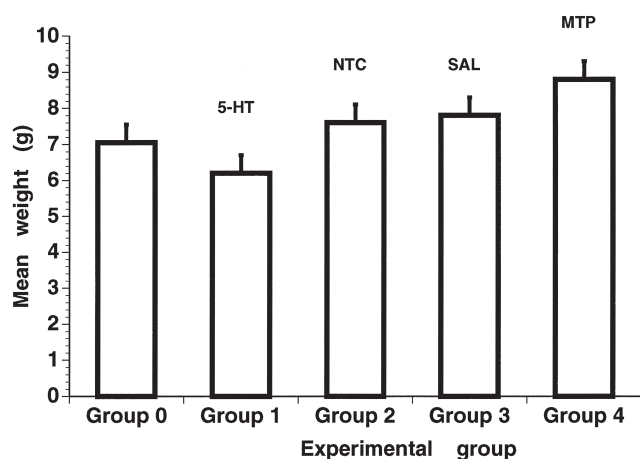


Fig. 1: Body weight of adult females of *Aphonopelma hentzi* after a 10-week feeding period. Experimental groups ( $n=15$ /group) consisted of non-treated controls (NTC, Group 2), and spiders injected with serotonin (5-HT, Group 1), physiological saline (SAL, Group 3), and methyltryptophan (MTP, Group 4), a serotonin antagonist. Group 0 represents the mean weight for all spiders at the beginning of the experiments. Data are expressed as means; vertical lines represent  $\pm$  SD. See text for details.

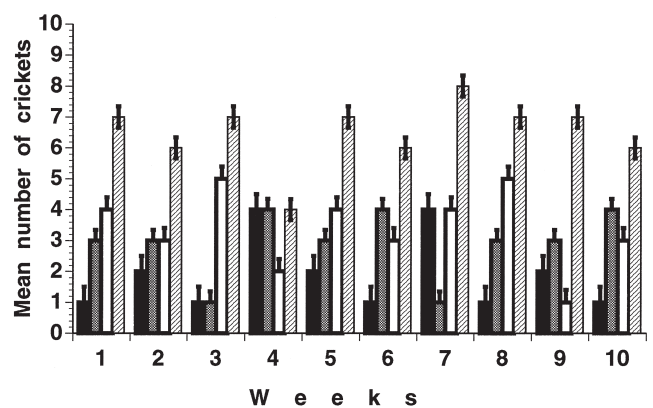


Fig. 2: Number of crickets eaten per week by adult females of *Aphonopelma hentzi* over a 10-week feeding period. Experimental groups ( $n=15$ /group) for each week, from left to right, consisted of spiders injected with serotonin (black bars), non-treated controls (stippled bars), and those injected with physiological saline (white bars) and methyltryptophan (hatched bars). Data are expressed as means; vertical bars represent  $\pm$  SD. See text for details.

spiders ( $p > 0.50$ ). There were significant differences between NTC spiders and those injected with 5-HT ( $p < 0.05$ ) and methyltryptophan (MTP) ( $p < 0.05$ ). There was also a significant difference between 5-HT and MTP spiders ( $p < 0.01$ ).

All adult females had an average weight of 7.05 g ( $\pm 0.15$  SD) at the beginning of the experiments, and NTC spiders exhibited a mean weight of 7.6 g ( $\pm 0.18$ ) after 10 weeks of feeding (a 7.8% weight increase). In contrast, spiders injected with 5-HT showed a weight loss of 12.9%, whereas those injected with MTP exhibited a 25.7% weight gain.

There was an overall significant effect of treatments on the number of crickets eaten per week (Fig. 2) ( $F = 20.17$ ,  $df = 9, 56$ ,  $p < 0.01$ ) over the 10-week period. No differences in food consumption were observed between NTC and SAL animals ( $p > 0.50$ ). Spiders injected with MTP ate significantly more crickets than both the NTC and SAL groups ( $p < 0.01$ ), whereas those injected with 5-HT ate significantly fewer crickets ( $p < 0.01$ ).

## Discussion

Various physiological mechanisms that may be involved in the regulation of dietary selection, diet balancing, and responses to hunger in arthropods have been postulated, including haemolymph sugar levels (Scriber & Slansky, 1981; Nakamura, 1987; Friedman *et al.*, 1991), chemoreceptors (Kennedy, 1987; Simpson & Simpson, 1990), gut volume (Anderson, 1974; Nakamura, 1987; Foelix, 1996; Punzo, 1989a, b, 1995, 2000), and neurotransmitters/neuromodulators (Fernstrom & Wurtman, 1971; Cohen *et al.*, 1988). A recent study by Cohen (2001) showed that injection of 5-HT led to a decrease in overall feeding as well as an increase in carbohydrate intake in insects. The results of the current experiment suggest that this serotonin-feeding relationship occurs in spiders as well. The manipulation of 5-HT levels caused adults of *A. hentzi* to alter their feeding behaviour. In addition, the administration of MTP, a serotonin antagonist, caused an increase in the amount of prey consumed and body mass.

It remains to be determined what the proximate mechanisms are by which 5-HT alters feeding rates in arthropods and other animals. Do decreasing haemolymph sugar levels associated with hunger cause concomitant changes in 5-HT concentrations in the brain and haemolymph? Does 5-HT act as a neurotransmitter in neural pathways within the CNS or in peripheral nerve fibres? Does it act as a neuromodulator or neurohormone affecting electrogenic events in specific brain regions associated with feeding and the perception of satiation? Future studies should address these questions in an attempt to understand more fully the proximate factors associated with the regulation of feeding behaviour and/or diet selection in arthropods.

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