unusual. All specimens were found in two small chambers at the back of the cave. These chambers are in the upper part of the cave and therefore less humid. The soil is dusty. The description of the biotope of the Italian *Larca italica* provided by Gardini (1983) is similar. *Larca* species in general seem to prefer the dry and upper parts of their habitats.

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Possible role of brain monoamines in the dispersal behaviour of spiderlings of *Hogna carolinensis* (Walckenaer) (Araneae, Lycosidae)

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Summary

Experiments were conducted to identify neurochemical parameters (changes in brain monoamines) associated with the dispersal of spiderlings of *Hogna carolinensis* (Lycosidae). Brain weights did not differ over the five-day period immediately after emergence from the egg sac, during which the spiderlings remained attached to the abdomen of the maternal parent. All spiderlings dispersed from their mothers on day 6 after emergence (under rearing conditions of 23°C, 70% relative humidity, and a 14L:10D photoperiod regime). Mean brain concentrations of serotonin (5-HT) ranged from 56.61–62.23 nm/mg for spiderlings from days one to five after emergence, the time during which they remained with their mother, and increased to 81.34 nm/mg on day 6 (following dispersal), and 81.13 nm/mg for adults. There was no significant difference in 5-HT levels between the brains of adult males and females, or between 6-day old spiderlings and adults. Levels of 5-HT increased significantly on day 6 after emergence as compared with concentrations recorded on day 5. There were no significant differences between mean brain concentrations of octopamine (OA) for days 1–5. However, on day 6, there was a significant increase in OA levels as compared with day 5. In contrast, dopamine (DA) concentrations did not change significantly at any developmental stage. The possible role of 5-HT and OA in the onset of dispersal behaviour in lycosid spiderlings is discussed.

Introduction

Biogenic amines (monoamines) are important neurotransmitters, neuromodulators, and neurohormones in the central nervous systems (CNS) of vertebrates and invertebrates. In numerous taxa they have been shown to play a role in the mediation of arousal and locomotor activity, short-term and chronic physiological stress, and aggressive and social dominance relationships (Bicker & Menzel, 1989; Haney *et al*., 1990; Corbet, 1991; Orchard *et al*., 1993; Punzo, 2001).

In arthropods, monoamines have been shown to play a role in the regulation of a variety of physiological responses as well as behaviour. Most of the research has been conducted on insects. For example, octopamine (OA) turnover rates increased significantly in crickets after fighting with conspecifics (Adamo *et al*., 1995). Significant increases in foraging and nest defence activities were associated with higher concentrations of OA, dopamine (DA), and serotonin (5-hydroxytryptamine, 5-HT) in the brain (supraoesophageal ganglion, SEG) of worker honeybees (Harris & Woodring, 1992). An increase in brain levels of 5-HT has been implicated in the onset of flight behaviour (dispersal) in boll weevils (Guerra *et al*., 1991).

The role of monoamines in the regulation of physiological parameters and behaviour in arachnids has received far less attention. Changes in monoamine concentrations in the SEG were associated with ontogenetic shifts in aggressive behaviour in solifugids (Punzo, 1998). The increased aggression exhibited by later nymphal instars was accompanied by significant changes in brain 5-HT and DA levels (Punzo, 1993,

1994). Brain serotonin and OA levels were significantly reduced 30 min after staged encounters between conspecific males of the theraphosid spider *Aphonopelma hentzi* (Girard) in both dominant and subordinate individuals as compared with isolated controls (Punzo & Punzo, 2001). Agonistic interactions had no effect on the concentrations of DA. This was the first demonstration that the establishment of social status can cause changes in brain monoamines in spiders.

Wolf spiders (Lycosidae) present an interesting opportunity to assess the possible role of monoamines in the mediation of overall arousal and other specific behaviours. These spiders are vagrant hunters that forage over a wide variety of habitats in search of insects and other spiders (Shook, 1978; Gertsch, 1979; Nyffeler, 1999). Although cannibalism has been widely reported in this group (Hallander, 1970; Foelix, 1996; Samu *et al*., 1999), they are also known for their maternal care of young (Eason, 1964; Higashi & Rovner, 1975). Several weeks after the construction of an egg sac, female lycosids tear the outer casing of the egg sac with their fangs and remain motionless while the spiderlings emerge and climb onto her abdomen forming a clustered mass (Rovner *et al*., 1973). They remain with their mother until their yolk supply is depleted, a period that can last up to one week, depending on the species. Following this period, they crawl to the surface of the ground and disperse.

It is well known that food deprivation (increased hunger level) is associated with an increase in locomotor activity in many animals, including insects (Bernays & Simpson, 1982), spiders (Foelix, 1996; Punzo, 2000a), solifugids (Punzo, 1997, 2000b), and decapod crustaceans (Kravitz, 1988). Although the decision to disperse from their mother may be closely associated with the depletion of yolk reserves and increasing hunger levels in lycosid spiderlings, it may also be mediated to some extent by changes in brain monoamine levels. This is supported by Guerra *et al*. (1991) who found that the onset of dispersal and flight behaviour in boll weevils (Coleoptera) was accompanied by significant increases in brain serotonin levels independent of hunger levels.

The purpose of the present study was to determine what changes, if any, in brain monoamine concentrations are associated with postembryonic development in spiderlings of the lycosid *Hogna carolinensis* (Walckenaer), from the time they emerge from the egg sac and climb onto the abdomen of their mother, to the time when they disperse.

Methods

Three adult female *Hogna carolinensis* carrying egg sacs were collected from different locations in Hillsborough and Pinellas Counties (Florida) during July 1999. They were brought back to the laboratory and maintained separately in plastic cages placed in a Precision Model 85 environmental chamber (Boone, Iowa) (23°C, 70% RH, and 14L: 10D photoperiod regime). They were provided with water *ad libitum*. None of the females fed when offered crickets and mealworms, but on several occasions they were observed drinking from cloth wicks saturated with water.

After emerging from their egg sacs, five spiderlings were selected at random and removed from each female on each of 6 consecutive days ($n=15$ spiderlings/day for each day, days 1–6). It was observed that all spiderlings dispersed from the female on day 6 under these rearing conditions. The total number of spiderlings carried by each adult female ranged from 236–310.

The spiderlings, as well as five different adults (3 females, 2 males; age=12 months), were placed individually in glass vials and preserved by freezing them at -40°C for subsequent neurochemical analysis. Before chemical testing, the specimens were thawed and the legs and abdomens were removed using iris microdissecting scissors (Carolina Biological Supply, Burlington, North Carolina). Brains were removed from the cephalothorax using a Unitron 750 dissecting microscope as previously described by Punzo (1983) and weighed on a Metler electronic analytical balance.

Brains were analysed for concentrations of 5-HT, OA and DA (monoamines that have been shown to function as neurotransmitters and neuromodulators in arachnids, see Meyer, 1991; Punzo & Punzo, 2001), according to the protocol described by Guerra *et al*. (1990) and Punzo (1994). To summarise, each brain sample was placed in a 2.0 ml centrifuge tube containing 250 μl of ice cold 0.1N perchloric acid that contained 40 ng of 3-methoxy-4-hydroxyphenethyl alcohol (MOPET) as an internal standard and 1.0 mM sodium metabisulfite. The sample was sonicated and then centrifuged at 15,000 g for 2 min in a Sorvall Model 100A high speed centrifuge. Serotonin was determined using high performance liquid chromatography with electrochemical detection (HPLC-ED, Beckman Model 47A). Following centrifugation, 20 μl of supernatant were injected directly into the HPLC column (40 cm in length, with a 0.2 μ pore diameter) packed with Hypersil and provided with a Hewlett-Packard 760E detector (0.40 V). The mobile phase (flow rate, 3000 psi) used to elute the monoamines consisted of 20 mM sodium acetate, 12% acetonitrile, 100 mM sodium dihydrogen orthophosphate, 2.5 mM octane sulfonic acid, and 0.3 mN EDTA disodium salt adjusted to pH 4.2 and filtered through a 0.45 μm filter. Each sample was compared with monoamine standards (5-HT, OA, DA) tested at the beginning of each assay and retested at 30-min intervals. The 5-HT, OA, and DA concentrations from the brains of the five adults as well as the spiderlings tested on days 1–6 were expressed as nmol/mg.

Statistical procedures followed those described by Sokal & Rohlf (1995). Data were analysed with an analysis of variance (ANOVA) using a completely randomised block design with postembryonic stages (treatments) arranged as factorials. Individual means for each day were compared using a Fisher's LSD test ($p<0.05$).

Results

Brain weights (wet weight) did not differ for spiderlings over the six-day period after emergence ($p>0.50$). They ranged from 0.871–0.924 mg (mean 0.881 mg ± 0.002 SD) on day one, to 0.832–1.12 mg (mean 0.909 mg ± 0.026) on day six.

Mean brain concentrations of 5-HT ranged from 56.61–62.23 nm/mg for spiderlings from days one to five after emergence, 81.34 nm/mg on day 6, and 81.13 nm/mg for adults (Fig. 1). There was no significant difference in serotonin levels between the brains of adult males and females, or between 6-day old spiderlings and adults ($p>0.50$). Levels of 5-HT increased significantly on day 6 after emergence as compared with concentrations recorded on day 5 ($p<0.05$).

There were no significant differences between mean brain concentrations of OA for days 1–5 (34.21–36.72 nm/mg; $p>0.50$). However, on day 6, there was a significant increase in OA levels (54.71 nm/mg) as compared with day 5 ($p<0.05$) (Fig. 1). In contrast, DA concentrations did not change significantly at any developmental stage.

Discussion

Spiderlings of *H. carolinensis* dispersed from their mother 6 days after emerging from their eggs sacs and climbing on her abdomen. This dispersal behaviour coincided with a dramatic increase in the concentrations of 5-HT and OA in their brains as compared with levels recorded for spiderlings on day five. Levels of DA, on the other hand, remained relatively constant during the maturation period from spiderling to adult. This suggests that, in addition to the depletion of yolk reserves, the onset of dispersal behaviour by lycosid spiderlings might be controlled by a more complex set of proximate factors involving 5-HT and OA, which have been shown to function as neurotransmitters, neuromodulators and/or neurohormones in arthropods with concomitant effects on physiology and behaviour (Murdock & Oman, 1981; Kravitz, 1988; Meyer, 1991; Orchard *et al.*, 1993; Punzo, 1993, 1996; Yeh *et al.*, 1996).

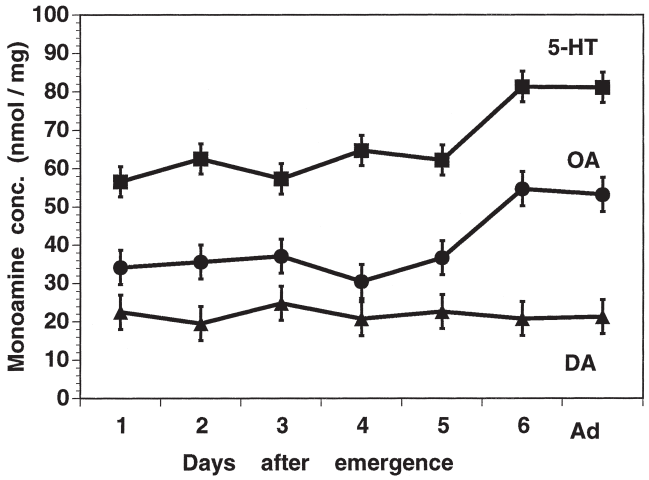

Fig. 1: Concentrations (in nmol/mg) of monoamines from the brains of spiderlings (assayed on days 1–6 after emergence from egg sac) and adults (Ad) of *Hogna carolinensis*. Data expressed as means ($n=5$ for each day). Vertical bars represent ± SD. Squares = serotonin (5-HT); circles = octopamine (OA); triangles = dopamine (DA). All monoamine concentrations on days 1–5 were not significantly different ($p>0.50$); 5-HT and OA levels on day 6 were significantly different from those on day 5 ($p<0.05$). See text for details.

This is in agreement with the few previous studies on the relationship between CNS monoamine concentrations and specific behaviours in arthropods. For example, a significant increase in brain serotonin was observed to coincide with the onset of locomotor activity in crickets (Cymborowski, 1970), and with the dispersal behaviour and flight of boll weevils from tropical and temperate regions, regardless of foods consumed (Guerra *et al.*, 1991). Changes in brain monoamine levels have been associated with learning and memory (Bicker & Menzel, 1989; Lin & Roelofs, 1992; Punzo, 1996), and have been implicated in a variety of ontogenetic shifts in behaviour (caste polytheism) in honeybee workers, including discrimination between different sets of olfactory cues (Macmillan & Mercer, 1987) and the onset of nest-guarding behaviour (Moore *et al.*, 1987). It should also be pointed out that a clear relationship has been established between social dominance relationships, stress, aggression and dispersal behaviour in vertebrates, with an increase in the utilisation of 5-HT in various brain regions (Mason, 1986; Haney *et al.*, 1990; Winberg *et al.*, 1997; Punzo, 2001). In insects, it has been suggested that OA, which appears to function in many similar ways to norepinephrine in the CNS of vertebrates (Downer & Hiripi, 1993), is part of a general arousal system which prepares these animals for a variety of vigorous skeleto-muscular activities, territorial defence, and stress (Corbet, 1991; Orchard *et al.*, 1993). The results reported here for *H. carolinensis* suggest that it may play a similar role in spiders.

Although only a few studies exist, it has been demonstrated that changes in the concentrations of CNS monoamines are associated with the onset of certain behaviours in arachnids as well. For example, in many solifugids, including the eremobatid *Eremobates palpisetulosus* Fichter, newly hatched postembryos are gregarious, do not feed, and are essentially immobile (Punzo, 1998). First-instar nymphs resemble adults in general appearance and increase their locomotor activity. During the second nymphal instar, solifugids begin to hunt and feed, burrow, and become increasingly agonistic toward conspecifics. This increase in aggression is accompanied by a significant increase in 5-HT levels in the brain (Punzo, 1994).

A more recent study showed that brain 5-HT and OA levels decreased significantly within 30 min after male-male fighting in the tarantula *Aphonopelma hentzi*, as compared with isolated controls (Punzo & Punzo, 2001). In addition, 5-HT and OA concentrations were significantly lower in subordinate (losing) vs. dominant (winning) spiders. Agonistic interactions had no effect on DA levels.

In conclusion, the results of this study indicate that the dispersal of lycosid spiderlings from their maternal parent is accompanied by a significant increase in the concentrations of 5-HT and OA in the brain. Thus, in addition to the depletion of yolk reserves, CNS monoamines may play an important synergistic role in the onset of locomotor activities associated with dispersal and subsequent foraging. Future studies should focus on identifying possible neuroarchitectural changes in various brain regions that may account in some way for the observed changes in neurochemistry.

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