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ORIGINAL ARTICLE

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Contact with maternal parent and siblings affects hunting behavior, learning, and central nervous system development in spiderlings of *Hogna carolinensis* (Araeneae: Lycosidae)

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Abstract The purpose of this study was to determine the effects of early experience (rearing conditions) on the central nervous system (CNS) and behavior of spiderlings of *Hogna carolinensis* (Lycosidae). We were interested in whether or not spiderlings that were allowed to remain in contact with their maternal parent and siblings (enriched condition, EC) would exhibit differences in CNS development or subsequent behavior when compared with those reared in isolation (impoverished condition, IC). Spiderlings emerged from their egg sacs and climbed onto the dorsal surface of their mother's abdomen where they remained until their yolk supply was depleted (5 days). They dispersed on day 6 after emergence. We compared the ability of 16-day-old EC and IC spiderlings to capture prey in a linear runway and to learn a complex maze (spatial learning). We also compared certain aspects of CNS development (brain weight, total number of brain cells, volume of central body and protocerebral neuropil) in EC and IC spiderlings. Results indicated that EC subjects are more efficient at capturing moving prey (crickets) and exhibited improved performance (significantly fewer blind alley errors) in the maze. The volume of the protocerebral neuropil in 6-day-old EC animals increased 30% over a 5-day period after emergence as compared to IC animals of the same age. The volume of the central body of EC animals increased 34.8% over the same time period. On day 6 after emergence, the weight of the protocerebrum was significantly greater in EC versus IC subjects. There were no significant effects of rearing condition (EC vs IC) or age (1- and 6-day-old spiderlings) on the total number of nerve cells in the protocerebrum, suggesting that the difference in protocerebral weight was due primarily to differences in supporting glial tissues and neuropil matrix. In conclusion, the data suggest that early contact with the maternal parent and siblings is of vital importance to CNS development in lycosid spiderlings and can influence the capacity for spatial learning as well as the ability to capture prey.

Keywords Early experience · *Hogna carolinensis* · Hunting behavior · Maternal contact · Rearing condition

Introduction

Although it is known that environmental factors such as ambient temperature and moisture can influence many physiological, morphological, and developmental processes in ectotherms that may have profound effects on phenotypic traits later in life (Burger 1991; Punzo 1991, 2000a), there is little information on the effects of early experience on central nervous system (CNS) development and subsequent behavior in invertebrates, including arthropods. In fact, as recently as 1985, many researchers held the view that the development of the invertebrate CNS was so rigidly programmed as to render it qualitatively different from those of vertebrates (Easter et al. 1985), which were considered to exhibit a higher degree of plasticity and whose patterns of neural connections were more directly responsive to early experience (Rosenzweig and Bennett 1996; Nilsson et al. 1999; Smulders et al. 2000; Punzo 2001).

However, there have been some studies on arthropods that have forced a re-evaluation of this view, showing that CNS neurons may exhibit prolonged periods of malleability and that environmental complexity can influence neurogenesis, synaptogenesis, and the degree of dendritic proliferation. For example, significant changes in the number and spinal morphology of neurons in the brains of newly emerged honeybees (*Apis mellifera*) occurred after their first orientation flight from the nest (Coss and Brandon 1982). More recently, Lomassese et al. (2000) reported that the brains of crickets (*Acheta domesticus*)

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reared in enriched environments (exposure to stimuli from conspecifics) contained more neurons than those exposed to impoverished conditions during early life.

The effects of early experience/rearing conditions on the subsequent behavior of arthropods are poorly understood, especially with respect to learning. Learning represents an experience-dependent modification of enduring internal representations of the external environment (Dudai 1989). The degree to which an animal is able to modify appetitive behaviors in response to alterations in physiological state (motivation) and changing environmental conditions can increase survivorship (Mangel 1993; Punzo 1996). Although the behavior of arthropods has long been regarded as relatively inflexible (Papaj 1993), there have been numerous studies (emphasizing insects) showing that their behavior can be modified based on past experience (see reviews by Lahue 1973; Punzo 1985, 1996; Papaj and Lewis 1993; Menzel et al. 1997). In addition, the ability of an animal to learn specific locations associated with food or escape routes (spatial learning) can result in an optimization of foraging and increased fitness (Olton 1978; Gallistel 1990).

Given the paucity of data on the general cognitive capacities of spiders (Punzo 1996, 2000b, 2002; Heiling and Herberstein 1999; Rodriguez and Gamboa 2000; Venner et al. 2000) we conducted experiments to determine the effects of early experience on CNS development and subsequent behavior. We chose the wolf spider, *Hogna carolinensis* Walckenauer (Lycosidae) (formerly *Lycosa carolinensis*), for a number of reasons. Firstly, it is a large, commonly occurring species that ranges over a wide geographic area in the United States (Gertsch 1979) and is easy to rear and maintain in captivity. Secondly, its natural history and life cycle are fairly well known (Shook 1978; Foelix 1996). Thirdly, lycosid females typically guard their egg sacs and carry them about when moving from place to place (Eason 1964). When the spiderlings are ready to emerge, the female tears the outer casing of the egg sac with her fangs and remains motionless while the spiderlings climb onto the dorsal surface of her abdomen, forming a clustered mass (Rovner et al. 1973; Nyffeler 2000). They remain with their mother until their yolk supply is depleted, a period that can last up to 1 week. Following this period, they crawl to the surface of the ground and disperse (Higashi and Rovner 1975). Thus, lycosids allow one the opportunity to investigate the effects (if any) of maternal care and contact with siblings (rearing conditions) on the subsequent behavior of spiderlings.

In this study we investigated the effects of contact with the maternal parent and siblings on certain aspects of CNS development as well as on the subsequent hunting behavior and spatial learning ability of spiderlings of *H. carolinensis*. To our knowledge, no previous data exist on the effects of rearing conditions on these parameters for spiders.

Methods

Subjects

Forty females of *H. carolinensis* carrying egg sacs were collected from different locations in Hillsborough and Pinellas Counties (Florida, USA) during June and July 1999. They were transported back to the laboratory and maintained separately in plastic cages placed in Percival Model 85 environmental chambers (Boone, Iowa, USA) at 23±1°C, 68–74% relative humidity, and a 14L:10D photoperiod regime. They were provided with water ad libitum and fed on a diet of crickets (*A. domesticus*) and mealworms (*Tenebrio molitor*). However, only six of the females accepted any food items; the rest did not feed until after the spiderlings had emerged from the egg sacs. It is common for female lycosids to stop feeding while carrying egg sacs (Gertsch 1979). The number of spiderlings that emerged ranged from 184–288 per sac. Under these laboratory conditions, spiderlings remained with their maternal parent for a period of 5 days, dispersing on day 6 after emergence.

Rearing conditions

After emergence, 50 spiderlings were selected at random from each of 10 egg sacs (also selected at random from the 40 egg sacs originally collected), yielding a potential population of 500 subjects. These 500 subjects were randomly assigned to one of two groups (n=250/group). In one group, 25 spiderlings were allowed to remain with their natural maternal parent (n=10 females; 25 spiderlings/female, n=250) until they dispersed on day 6 after emergence. In this way, they were exposed to a variety of olfactory and tactile cues as a result of contact with their mother and siblings (enriched condition, EC). Spiderlings from the other group emerged from egg sacs that had been removed from the adults after being deposited. Emergent spiderlings were then reared in isolation and thus had no contact with a maternal parent or siblings (impoverished condition, IC).

After dispersal, all of the EC spiderlings were housed individually in plastic vials (3 cm diameter; 11 cm length) and maintained under identical environmental conditions as those described previously for the adults. They were provided with water ad libitum and fed three times/week on a diet of apterous *Drosophila melanogaster* and small cricket nymphs (*A. domesticus*). All subjects were maintained in this way for 2 weeks before the onset of behavioral testing and neurological analyses. The subjects from the IC group were housed and maintained in the same way. They were fed on the same type of diet starting on day 6 after emergence (when yolk supplies are depleted). The spiderlings used in these experiments were all of similar size (body length: 4.5–4.8 mm).

Procedures for behavioral tests

Hunting behavior

This experiment was conducted to ascertain the effects (if any) of rearing conditions (early experience) on the ability of 16-day-old spiderlings to capture prey using the runway apparatus shown in Fig. 1. Prey consisted of small nymphs of *A. domesticus* of similar size (body length: 2.2–2.3 mm). The runway was constructed of white vinyl panels. There was a lateral compartment adjacent to the runway that housed the spiderling (predator) (Fig. 1, S) and a compartment at one end of the runway that housed the prey (Fig. 1, P). The prey compartment contained an intake valve (v) through which compressed air was delivered to motivate the prey to move rapidly down the runway toward the spiderling. This had proved quite successful in previous experiments on prey capture by theraphosid spiders (Punzo 1989). There was a sliding door (d) that prevented the prey from entering the runway until removed. Two photoelectric cells (p) (Thornton Co., Chicago, Ill.) were placed 4.0 cm

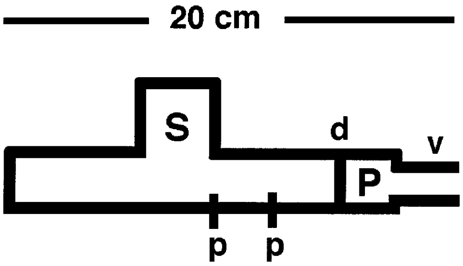

Fig. 1 Diagrammatic representation of apparatus used to determine prey capture success by spiderlings of *Hogna carolinensis*. *P* location of prey; *d* sliding panel door; *S* location of spider; *v* air valve; *p* photoelectric cells. See text for details

apart immediately adjacent to the open end of the predator compartment (S). These cells were connected to a Simpson Model HK electronic timer. As soon as a cricket passed the proximal photoelectric cell the timer was automatically activated. When the cricket passed the second (distal) cell immediately adjacent to the opening of the predator compartment, the timer was stopped. This allowed us to determine the running speed of the prey (centimeters per second) (Punzo 1989). Cricket running speeds ranged from 0.5 to 2.9 cm/s. Only trials in which the crickets ran at a fairly consistent and rapid rate (2.4–2.5 cm/s) were used in data analyses. A cool fluorescent lamp was positioned directly over the center of the runway to provide lighting. All subjects were deprived of food for 48 h prior to testing. Previous pilot studies had indicated that this food deprivation period provides sufficient motivation for seizing and ingesting prey (unpublished data).

At the beginning of each trial, an individual spiderling was removed from its home vial and transferred to a small glass vial (1.5 mm diameter). The vial containing the subject was placed in a compartment adjacent to the main runway (Fig. 1, S) with its open end on the floor. The vial was oriented in such a way that the subject was facing the runway floor with its front legs in contact with the edge of the runway. In addition, the prey item (a single *A. domestica* nymph) was placed in compartment P whose sliding door (d) was closed, preventing the cricket from entering the runway. The spiderling and cricket were allowed to remain in their respective compartments for 5 min before the start of a trial. The door of the prey compartment was then removed and a puff of air delivered to the tip of the cricket's abdomen. This caused the cricket immediately to begin to run along the length of the runway.

Thirty spiderlings were randomly selected from each group (EC and IC) (*n*=60) and received five trials/day over a 6-day period. For each trial we recorded whether the cricket was captured (considered a “correct” response) or struck at and not seized, thereby allowing the cricket to pass unharmed (considered an “error”). Trials in which the spiderling did not respond at all to the passing prey occurred infrequently (<1%) and were not used in data analyses. The walls and floor of the runway were washed with a dilute ammonia solution after each trial. All statistical procedures followed those described by Sokal and Rohlf (1995). Bartlett's test for homogeneity indicated that the data fit the assumptions of parametric procedures. The total number of errors made by each subject were tabulated and analyzed using a *t* test with the null hypothesis that rearing condition had no effect on prey-capture ability.

Spatial learning

This experiment was conducted to determine if rearing condition influenced the spatial learning ability of *H. carolinensis* spiderlings using a complex maze (Fig. 2). Learning experiments were conducted in two replications with identical testing procedures used for each replication. Previously untested 16-day-old spiderlings were used in these experiments.

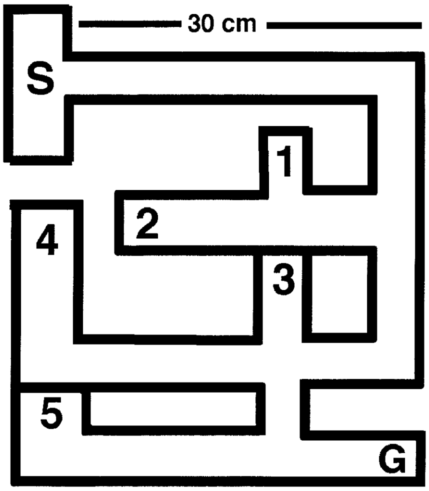

Fig. 2 Maze used to assess spatial learning ability of spiderlings of *H. carolinensis*. The maze contained a start box (*S*) and goal box (*G*), and five blind alleys. See text for details

The floor of the maze consisted of a piece of vinyl sheeting attached to a plywood base. The walls of the maze consisted of vinyl panels. The floor design of this five-blind-alley maze was originally used by Turner (1913) with the cockroach *Perpilaneta americana* and the maze has been enlarged for use with a variety of mammals (Gormezano and Wasserman 1992). The maze contained a start box (S) and a goal box (G). A General Electric 150-W light was positioned directly over the center of the maze that emitted enough heat so that the floor of the maze had an ambient temperature of 35°C. This temperature is below the lethal temperature for these spiderlings (42.7°C, unpublished data). However, previous pilot studies have shown that this combination of temperature and bright light provided sufficient motivation for these spiderlings to move actively throughout the maze. The goal box was covered with a wooden lid and its walls insulated with cork panels. As a result, the ambient temperature inside the goal box was reduced to a non-stressful temperature (28±0.2°C), and the wooden lid also allowed the subject to escape the aversive bright light. The start box was provided with a movable restraining panel so that the subject was prevented from entering the main body of the maze until the panel was removed.

As in the previously described runway experiments, all spiderlings were deprived of food for 48 h prior to testing. At the beginning of each trial a spiderling was removed from its housing vial, placed in a small glass vial, and transferred to the start box where it was allowed to remain for 3 min prior to the start of a trial. The panel was removed and the subject allowed to enter the body of the maze.

Six EC and 5 IC animals chosen at random were tested in the first replication, and 13 EC and 13 IC subjects in the second replication. During the second replication, 5 EC animals died during the days they were being tested, yielding a total number of 8 EC subjects for that replication. Each subject received ten trials/day over a 10-day training period. Learning trials were conducted between 1600 and 2100 hours based on peak periods of activity observed for this species in the field (personal observation). We recorded the number of blind-alley errors made by each subject for every trial and determined the total number of errors (total error score) made by EC and IC subjects over the 10-day training period. An error was scored when at least one-half of an animal's body entered a blind alley as defined by Rabinovitch and Rosvold (1951). Using a stopwatch we also recorded the latency (in seconds) required for the subjects to leave the start box and enter the main body of the maze (start box time), and run time (time that elapsed from leaving the start box to entering the goal box) for each trial. A repeated measures ANOVA was used to test for the effects of days of training on performance of EC and IC spiderlings in the maze. An arcsine transformation was performed on the data to correct for heterogeneity of variance.

Neurological analyses

The prosomal nerve mass of spiders consists of the supraesophageal ganglion (SEG) or brain, and the subesophageal ganglionic mass (Fig. 3). The SEG is considered to be the brain region most associated with the mediation of complex behaviors in arthropods, including learning and memory in insects (Punzo 1996; Heisenberg 1998) and spiders (Punzo 1988a). It consists of the protocerebrum (PC) containing a marginal layer of neurons (cortex) and a central mass of nerve fibers called the neuropil where most synaptic connections occur (Foelix 1996), and the cheliceral ganglia. The PC contains several association centers including the central body (Fig. 3, CB), mushroom bodies, and several optic tracts (Fig. 3, Alen, Amen, Pmen, Pmol) innervating the median and lateral eyes. At least one optic tract connects with the CB. The globuli cells (Gb), also found in the PC, contain axon bundles (neurites) that form the so-called glomeruli (Glm). The axons of the globuli cells and those of other cell types form a fibrous neuropil called the haft (hf) in each half of the PC. Each haft extends mid-centrally, eventually forming a cluster of fine fibers called the bridge (Brg). The Gb, Glm, hf, and Brg together make up the so-called corpora pedunculata or mushroom bodies (MB) of the spider brain. For detailed descriptions of the arachnid CNS the reader is referred to several reviews (Bullock and Horridge 1965; Babu and Barth 1984; Foelix 1996; Punzo 1998).

The SEG was examined for neurological analyses for spiderlings at various ages reared under EC and IC conditions. The brains were removed in saline using microdissection scissors and weighed to the nearest 0.01 mg on a Sartorius Model 220B electronic analytical balance (Chicago, Ill.). The PC from 20 spiderlings (at 1 and 6 days after emergence from the egg sac) for each of the EC and IC groups (n=80) was separated from the rest of the prosomal nerve mass as described by Punzo (1988b). The PC was fixed immediately for 24 h in Bouin's solution. Fixed brains were then dehydrated through a series of ethanols, cleared in toluene, and embedded in Paraplast (Oxford Labware, St. Louis, Mo.) as described by Pantin (1964).

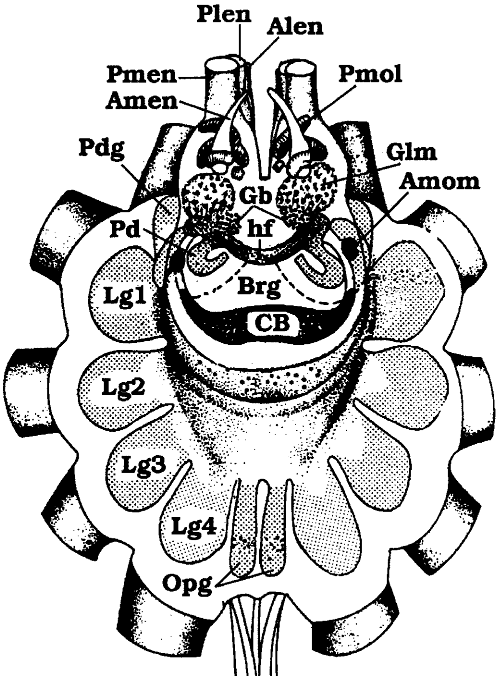

Fig. 3 Diagram of the various regions of the brain (dorsal view) of a spider such as *H. carolinensis* (modified after Babu and Barth 1984). *Alen* anterior lateral eye; *Amen* anterior median eye; *Amom* anterior median optic mass; *Brg* neural bridge; *CB* central body; *Gb* globuli cells; *Glm* glomeruli; *hf* haft; *Lg1–4* leg ganglia for legs 1–4; *Opg* opisthosomal ganglion; *Pd* peduncle; *Pdg* pedipalpal ganglion; *Plen* posterior lateral eye nerve; *Pmen* posterior median eye nerve; *Pmol* posterior median optic lamella

For nerve cell counts and volume measurements the brains were sectioned in the transverse plane at 8 μm using a microtome (Stoelting Model 56753, Wood Dale, Ill.) and stained via the Feulgen reaction (Humason 1979) by using a commercially prepared Schiff reagent (S5133, Sigma Chemical Co., St. Louis, Mo.). Other tissue sections were stained using the Golgi rapid method (Strausfeld 1980), Casson's trichrome stain as described by Kiernan (1990), or Palmgren's silver technique (Palmgren 1948). Brain volume measurements were determined using a microscope fitted with an ocular micrometer as described by Babu (1975).

We used the photomicrographic method (Cayre et al. 1996) to estimate the total nerve cell counts in the brains of EC and IC animals at 1 and 6 days of age. No attempt was made to distinguish between the various cell types that have been described for some spiders (Babu and Barth 1984). To summarize, we used photomicrographs of horizontal and transverse serial sections at known magnification taken with a Unitron Model 657 microscope. Nerve cells were estimated in various brain regions using unbiased stereologic techniques as described by West (1999) to ensure that all tissue regions had an equal probability of being sampled. The total number of cells for complete serial reconstructions was estimated using a combination of the Cavalieri method of volume estimation (Coggeshall and Lekan 1996) and the optical dissector direct-counting method (West 1999).

ANOVAs (2×2 factorial), with rearing condition (EC, IC) and age (1, 6 days) as the factors, were used to assess the effects of these parameters on number of cells, volume of protocerebral neuropil, and volume of CB, and t tests were used where appropriate to test for significance between means.

Results

Hunting behavior

This experiment attempted to determine if contact with the maternal parent and siblings (rearing condition) had any effect on the ability of these spiderlings to capture prey. Spiderlings from the EC group were more efficient at capturing prey, making an average of 9.4 errors (range: 4–13; ±1.12 SE), which was significantly fewer than the 15.3 errors averaged by the IC group (8–23; ±2.21 SE) (t_{58}=3.16, P<0.01).

Spatial learning

This experiment attempted to ascertain if rearing condition had an effect on performance in a spatial learning task. The results show that the performance of both EC and IC subjects in this maze improved significantly over the training period (Fig. 4). Total error scores during maze testing served as the measure of performance between EC and IC subjects, a commonly used criterion in complex maze studies (Yehuda et al. 1988). EC subjects made a total of 552.7 errors over the 10-day period as compared to

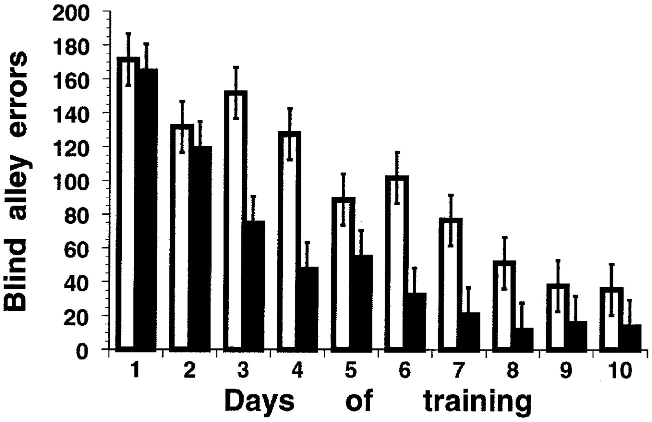

Fig. 4 Number of blind alley errors made by spiderlings of *H. carolinensis* in a complex maze over a 10-day training period. *Open bars* subjects reared in isolation (impoverished condition); *solid bars* subjects allowed contact with their maternal parent and siblings (enriched condition). Values are expressed as means; *vertical lines* represent ±SE. See text for details

Table 1 Start box time (latency in seconds for a spiderling to leave the start box and enter the main body of the maze) and run time (elapsed time from leaving the start box to entering the goal box) for spiderlings of *Hogna carolinensis* in a complex maze (on day 10). Spiderlings reared under enriched conditions were allowed contact with their maternal parent and siblings while those under impoverished conditions were reared in isolation. Data expressed as means (±SE) summed over all trials. Values followed by different superscripts are significantly different ($P<0.01$)

Group	*n*	Start box time (s)	Run time (s)
Enriched conditions	14	47.4±5.7^{a}	170.3±11.6^{a}
Impoverished conditions	18	137.7±9.4^{b}	224.6±14.1^{b}

972.7 for the IC group. The ANOVA showed an overall significant effect of rearing condition ($F_{1,28}$=9.82, P<0.005) and days of training ($F_{9,252}$=24.11, P<0.001) on performance.

The results listed in Table 1 indicate that EC subjects left the start box considerably earlier than IC spiderlings as confirmed by a significant group (EC vs IC) main effect ($F_{1,28}$=18.45, P<0.01) performed on the logarithmically transformed start box latency data. In addition, IC subjects spent considerably more time running the maze (Table 1) as compared to EC subjects ($F_{1,28}$=14.32, P<0.01).

Neurological correlates

The results of the neurological analyses are shown in Table 2. There were no significant effects of rearing condition ($F_{1,19}$=0.47, P>0.54) or age ($F_{1,19}$=0.25, P>0.47) on the total number of nerve cells in the PC of these spiderlings.

There was no difference in the volume of the protocerebral neuropil between IC subjects over the 6-day period after emergence from the egg sac (P>0.57). However, in EC subjects, the volume increased 30% between days 1 and 6 (t_{19}=2.32, P<0.05). A similar profile was observed for the volume of the CB. The CBs of IC animals did not significantly change in volume between day 1 and 6 (P>0.61). In EC animals, however, the volume of the CB increased 34.8% over the same time period (t_{19}=2.53, P<0.05).

Table 2 Body weight, nerve cell counts, and brain volume measurements from various regions of the protocerebrum for spiderlings of *H. carolinensis*. Age of spiderlings given in days after emergence from egg sac. Values expressed as means±SD. Twenty subjects were analyzed at each age interval and rearing condition. *EC* spiderlings reared under enriched conditions; *IC* impoverished conditions. See text for details

Parameter measured/ brain region	Age of spiderlings	
	Day 1	Day 6
Number of nerve cells		
IC protocerebrum	37223.4±824.8	38121.7±1037.5
EC protocerebrum	38006.3±1003.1	38216.8±974.6
Volume of protocerebral neuropil (mm^3)		
IC animals	0.259±0.03	0.249±0.01
EC animals	0.262±0.01	0.372±0.02
Volume of central body (mm^3)		
IC animals	0.051±0.01	0.058±0.02
EC animals	0.053±0.02	0.089±0.01
Weight of protocerebrum (mg)		
IC animals	0.212±0.02	0.237±0.03
EC animals	0.237±0.01	0.359±0.02
Body weight (mg)		
IC animals	13.7±1.2	15.2±0.9
EC animals	13.2±0.4	14.7±1.3

Rearing condition had an effect on the weight of the PC in spiderlings at 6 days after emergence. On day 6, the protocerebral weight of EC subjects had increased almost 40% as compared to only 10.5% for the IC group (t_{19}=2.71, P<0.05). The weights between EC and IC subjects did not differ on day 1.

Discussion

The results of this study clearly demonstrate that differences in the early experiences of spiderlings can have profound effects on their CNS development and subsequent behavior. Spiderlings reared under conditions where they were able to maintain contact with their mothers and siblings for 5 days after emergence from the egg sac, and hence were subjected to a variety of olfactory and tactile stimuli early in life, were more efficient at capturing prey and solving a spatial learning task than their counterparts who were reared in isolation (a condition of reduced environmental complexity). They also had heavier brains as well as a larger protocerebral neuropil and CB. To our knowledge, this is the first demonstration that maternal care and sibling interaction can influence CNS development and the subsequent behavior of offspring in spiders.

The ability to capture prey more efficiently and to remember the location of a food source or escape route (spatial learning) would certainly contribute to overall fitness. Thus, wolf spiderlings that remain with their mother before dispersal should exhibit a higher survivorship than those denied access to their mother. How frequently wolf spiderlings become dislodged from their mothers under natural conditions is not known. However, on several occasions we have observed spiderlings of *H. carolinensis* become dislodged from their mother in the field when she crawled into narrow rock crevices or brushed against low-hanging vegetation, resulting in a permanent separation for some (unpublished data). Flooding has been known to cause the separation of spiderlings from their mother (Rovner et al. 1973). Although Higashi and Rovner (1975) reported that wolf spiderlings (*Lycosa rabida*) that were forcefully separated from their mother readily drank water and survived as well as siblings that remained on the female, they did not investigate possible effects of separation from the mother on subsequent behavioral patterns. In contrast, Eason (1964) reported a higher mortality rate for spiderlings (*L. carolinensis*, *L. helluo*, *L. punctulata*, and *Schizocosa avida*) removed from their mother.

Spiderlings reared under conditions of enhanced environmental complexity were more adept at learning a complex maze (fewer total errors), an ability that would be advantageous in learning the locations of prey, shelter sites, and escape routes. Indeed, spatial learning tasks such as complex mazes represent ecologically relevant tasks for animals that move about or actively pursue prey (Davey 1989; Punzo 2002). In addition, EC spiderlings left the start box more rapidly, suggesting a higher level of exploratory behavior as compared to IC animals. EC spiderlings also required less time to reach the goal box. One way for an animal to optimize its foraging activities is to spend less time in random search patterns. The observation that EC animals had heavier brains with larger protocerebral neuropils and CBs is not surprising since the PC has been implicated in the mediation of complex behaviors in arthropods, including learning and memory, complex orientation movements, sound production, web spinning, territoriality and aggression, and nest construction, to name a few (Babu and Barth 1984; Punzo 1994, 1996; Foelix 1996; Heisenberg 1998; Punzo and Punzo 2002).

The results of this study also confirm a previous report on the effects of early experience on spiders showing that field-caught jumping spiders (*Phidippus audax*) were more active and less stereotactic in open-field tests than their captive-bred counterparts. In addition, spiders reared in cages provided with painted wooden dowels (providing enhanced novelty and more places to climb and hide) were more active and more adept at orienting toward prey than spiders reared in cages without dowels (Carducci and Jakobs 2000). In insects, which have received more attention, fruit flies (*D. melanogaster*) reared in darkness (visual deprivation) oriented differently toward visual targets than flies reared under a 12L:12D photoperiod regime (Hirsch et al. 1990). Host selection by some adult parasitoid insects can be influenced by odor cues experienced during larval stages (Turlings et al. 1993). Social insects (in this case ants) that were subjected to certain olfactory cues early in larval development exhibited different foraging behaviors as adults when compared to ants that had been reared in a different olfactory environment (Hölldobler and Wilson 1990). It would be interesting to study the effects of early experience on the subsequent behavior of social spiders, where numerous spiderlings remain in close proximity to adults and the offspring from other females. In these spiders, which construct communal nests, numerous individuals cooperate in hunting and nest maintenance activities (D'Andrea 1987). Would social spiders that were maintained in isolation from conspecifics as spiderlings exhibit different behavioral patterns when compared to those exposed to normal social interactions?

These experiments do not allow us to address which effect associated with early rearing conditions (contact with mother or siblings) is most significant. However, it should be pointed out that the spiderlings of many other species, including some orb-web and social spiders, remain in close contact with their siblings even though the risk of cannibalism can be high (D'Andrea 1987; Foelix 1996). Future studies should attempt to compare the relative importance of contact with a maternal parent versus contact with siblings for the subsequent behaviors and CNS development of spiderlings.

With respect to the CNS, enriched rearing conditions resulted in spiderlings having heavier brains and larger regions of the PC (neuropil and CB). In addition, these differences occurred in a relatively short period of time. It also suggests that the complexity of the environment experienced by spiderlings upon emergence can be of paramount importance to neurological development. Future studies should focus on cell counts in other regions of the CNS, including the optic tracts and tritocerebrum (which innervates the mouthparts), and also attempt to determine whether specific cell types in the brain respond differently to different rearing conditions. Finally, it would be useful to investigate the effects of enriched versus impoverished regimes on specific neurochemical parameters of the brain including the concentrations of neurotransmitters, neuromodulators, and neurohormones, all of which play an important role in the regulation of information processing in the CNS (Blenau and Erber 1998; Menzel 1999).

These aforementioned studies on arthropods are in agreement with the vast body of information available on the effects of environmental complexity on the development of the CNS in mammals and birds (see review by Rosenzweig and Bennett 1996). This work has shown that specific training procedures and/or differences in rearing conditions induced changes in the cerebral cortex with respect to its weight and thickness, number of synaptic connections and dendritic spines, degree of dendritic branching, and concentrations of DNA, low molecular weight neuropeptides, neurotransmitters, and neuromodulators. Similar changes have been reported for the hippocampus, which along with the cortex is involved in the mediation of learning, memory, and other complex behaviors. The results of this study further support the view that developmental plasticity of the CNS in response to experiential information is not limited to vertebrates and appears to have evolved early in the evolution of animal central nervous systems.

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References

Babu KS (1975) Post-embryonic development of the central nervous system of the spider, *Argiope aurantia* (Lucas). J Morphol 146:325–342

Babu KS, Barth FG (1984) Neuroanatomy of the central nervous system of the wandering spider, *Cupiennius salei* (Arachnida, Araneida). Zoomorphology 104:344–359

Blenau W, Erber J (1998) Behavioural pharmacology of the dopamine, serotonin and putative aminergic ligands in the mushroom bodies of the honeybee (*Apis mellifera*). Behav Brain Res 96:115–124

Bullock TH, Horridge A (1965) Structure and function in the nervous system of invertebrates, vol 2. Freeman, San Francisco

Burger J (1991) Effects of incubation temperature on behavior of hatchling pine snakes: implications for reptilian distribution. Behav Ecol Sociobiol 28:297–303

Carducci JP, Jakobs EM (2000) Rearing environment affects behavior of jumping spiders. Anim Behav 59:39–46

Cayre M, Strambi C, Charpin P, Augier R, Meyer MR, Edwards JS, Strambi A (1996) Neurogenesis in adult insect mushroom bodies. J Comp Neurol 371:300–310

Coggeshall RE, Lekan HA (1996) Methods for determining numbers of cells and synapses: a case for more uniform standards of review. J Comp Neurol 364:6–15

Coss RG, Brandon JG (1982) Rapid changes in dendritic spine morphology during the honeybee's first orientation flight. In: Breed MD, Michener CD, Evans HE (eds) The biology of social insects. Westview Press, Boulder, Colo., pp 338–342

D'Andrea M (1987) Social behavior in spiders (Asrachnida, Araneae). Monit Zool Italiano Monogr 3:1–156

Davey G (1989) Ecological learning theory. Routledge, London

Dudai Y (1989) The neurobiology of memory. Oxford University Press, Oxford

Eason RR (1964) Maternal care as exhibited by wolf spiders (lycosids). Proc Arkansas Acad Sci 18:13–19

Easter SS, Purver D, Rakic P, Spitzer NC (1985) The changing view of neuronal specificity. Science 230:507–511

Foelix RF (1996) Biology of spiders, 2nd edn. Oxford University Press, Oxford

Gallistel CR (1990) The organization of learning. MIT Press, Cambridge, Mass.

Gertsch WJ (1979) American spiders, 2nd edn. Van Nostrand, New York

Gormezano I, Wasserman EA (1992) Learning and memory. Erlbaum, Hillsdale, N.J.

Heiling AM, Herberstein ME (1999) The role of experience in web-building spiders (Araneidae). Anim Cogn 2:171–177

Heisenberg M (1998) What do the mushroom bodies do for the insect brain? Learn Mem 5:146–156

Higashi GA, Rovner JS (1975) Post-emergent behavior of juvenile lycosid spiders. Bull Br Arachnol Soc 3:113–119

Hirsch HVB, Potter D, Zawierucha D, Choudri T, Glasser A, Murphey RK, Byers D (1990) Rearing in darkness changes visually-guided behavior in *Drosophila*. Vis Neurosci 5:281–289

Hölldobler B, Wilson EO (1990) The ants. Harvard University Press, Cambridge, Mass.

Humason G (1979) Animal tissue techniques. Freeman, San Francisco

Kiernan J (1990) Histological and histochemical methods: theory and practice. Pergamon Press, New York

Lahue R (1973) The chelicerates. In: Corning WC, Dyal JA, Willows OD (eds) Invertebrate learning, vol 2. Arthropods and gastropod molluscs. Plenum Press, New York, pp 1–48

Lomassese SS, Strambi C, Charpin A, Augier R, Aouane AK, Cayre M (2000) Influence of environmental stimulation on neurogenesis in the adult insect brain. J Neurobiol 45:162–171

Mangel M (1993) Motivation, learning, and motivated learning. In: Papaj DR, Lewis AC (eds) Insect learning: ecological and evolutionary perspectives. Chapman and Hall, New York, pp 158–173

Menzel R (1999) Memory dynamics in the honeybee. J Comp Physiol [A] 185:323–340

Menzel R, Hammer M, Braun G, Sugawa M (1997) Neurobiology of learning and memory in bees. In: Goodman L, Fisher R (eds) The behavior and physiology of bees. CAB International, Berlin, pp 323–353

Nilsson M, Perfilieva E, Johansson U, Orwar O (1999) Enriched environment increases neurogenesis in the adult rat dentate gyrus and improves spatial memory. J Neurobiol 39:569–578

Nyffeler M (2000) Do adult female lycosids feed during the period of maternal care? Bull Br Arachnol Soc 11:388–390

Olton DS (1978) Characteristics of spatial memory. In: Hulse SH, Fowler H, Honig WK (eds) Cognitive processes in animal behavior. Erlbaum, Hillsdale, N.J., pp 341–373

Palmgren A (1948) A rapid method for selective silver staining of nerve fibers and nerve endings in mounted paraffin sections. Acta Zool 29:377–392

Pantin CF (1964) Notes on microscopal technique for zoologists. Cambridge University Press, London

Papaj DR (1993) Automatic behavior and the evolution of instinct. In: Papaj DR, Lewis AC (eds) Insect learning: ecological and evolutionary perspectives. Chapman and Hall, New York, pp 243–272

Papaj DR, Lewis AC (eds) (1993) Insect learning: ecological and evolutionary perspectives. Chapman and Hall, New York

Punzo F (1985) Recent advances in behavioral plasticity in insects and decapod crustaceans. Fla Entomol 68:89–104

Punzo F (1988a) Learning and localization of brain function in the tarantula spider, *Aphonopelma chalcodes* (Orthognatha, Theraphosidae). Comp Biochem Physiol A Physiol 89:465–470

Punzo F (1988b) Physiological amino acids in the central nervous system of the tarantulas *Aphonopelma chalcodes* and *Dugesiella echina* (Orthognatha, Theraphosidae). Comp Biochem Physiol C Pharmacol Toxicol Endocrinol 90:381–383

Punzo F (1989) Effect of hunger on prey capture and ingestion in *Dugesiella echina* Chamberlin (Orthognatha, Theraphosidae). Bull Br Arachnol Soc 8:72–79

Punzo F (1991) The effects of temperature and moisture on survival capacity, locomotor behavior, cuticular permeability, osmoregulation, and metabolism in *Centruroides hentzi* (Banks) (Scorpiones, Buthidae). Comp Biochem Physiol A Physiol 100: 833–837

Punzo F (1994) Changes in brain amine concentrations associated with postembryonic development in the solifugid, *Eremobates mormonus* (Roewer) (Solifugae, Eremobatidae). J Arachnol 22: 105

Punzo F (1996) Localization of brain function and neurochemical events associated with learning in insects. Rec Trends Comp Biochem Physiol 2:9–16

Punzo F (1998) The biology of camel-spiders (Arachnida, Solifugae). Kluwer, Norwell, Mass.

Punzo F (2000a) Desert arthropods: life history variations. Springer, Berlin Heidelberg New York

Punzo F (2000b) An experimental analysis of maze learning in the wolf spider *Trochosa parthenus* (Araneae: Lycosidae). Fla Sci 63:155–159

Punzo F (2001) Neurochemical correlates of agonistic interactions and dominance between males of the brown anole, *Anolis sagrei*. Fla Sci 64:131–139

Punzo F (2002) Reversal learning and complex maze learning in the spider *Aphonopelma hentzi* (Girard) (Araneae, Theraphosidae). Bull Br Arachnol Soc (in press)

Punzo F, Punzo TD (2002) Monoamines in the brain of tarantulas (*Aphonopelma hentzi*) (Araneae: Theraphosidae): differences associated with male agonistic interactions. J Arachnol 29:388–395

Rabinovitch MA, Rosvold HE (1951) A closed-field intelligence test for rats. Can J Psychol 5:122–128

Rodriguez RL, Gamboa E (2000) Memory of captured prey in three web spiders (Araneae: Araneidae, Linyphidae, Tetragnathidae). Anim Cogn 3:91–97

Rosenzweig MR, Bennett EL (1996) Psychobiology of plasticity: effects of training and experience on brains and behavior. Behav Brain Res 78:57–65

Rovner JS, Higashi GA, Foelix RF (1973) Maternal behavior in wolf spiders: the role of abdominal hairs. Science 182:1153–1155

Shook RS (1978) Ecology of the wolf spider, *Lycosa carolinensis* Walckenauer (Araneae: Lycosidae) in a desert community. J Arachnol 6:53–64

Smulders TV, Shiflett MW, Sperling AJ, DeVoogd TJ (2000) Seasonal changes in neuron numbers in the hippocampal formation of the food-hoarding bird: the black-capped chickadee. J Neurobiol 44:414–422

Sokal B, Rohlf FJ (1995) Biometry, 2nd edn. Freeman, New York

Strausfeld NJ (1980) The Golgi method: its application to the insect nervous system and the phenomenon of stochastic impregnation. In: Strausfeld NJ, Miller TA (eds) Neuroanatomical techniques: insect nervous system. Springer, Berlin Heidelberg New York, pp 132–190

Turlings TCJ, Wackers FL, Vet LEM, Lewis WJ, Tumlinson JH (1993) learning of host-finding cues by hymenopterous parasitoids. In: Papaj DR, Lewis AC (eds) Insect learning: ecological and evolutionary perspectives. Chapman and Hall, New York, pp 51–78

Turner CH (1913) Behavior of the roach (*Periplaneta americana*) in an open maze. Biol Bull 25:348–365

Venner S, Pasquet A, Leborgne R (2000) Web-building behavior in the orb-weaving spider *Zygiella x-notata*: influence of experience. Anim Behav 59:603–611

West MJ (1999) Stereological methods for estimating the total number of neurons and synapses: issues of precision and bias. Trends Neurosci 22:51–61

Yehuda R, McDonald D, Heller HG, Meyer J (1988) Maze-learning behavior in early adrenalectomized rats. Physiol Behav 44:373–381