

Habituation, avoidance learning, and spatial learning in the giant whipscorpion, *Mastigoproctus giganteus* (Lucas) (Arachnida, Uropygi)

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Summary

Studies were conducted on spatial learning in a T-maze (reversal learning) and complex maze, avoidance learning in a shuttle box, and habituation in the whipscorpion *Mastigoproctus giganteus*. The number of trials and errors required to reach the criterion (70% correct choices) for reversal learning in the T-maze decreased from means of 107.3 and 75.6, respectively (for the first reversal trial), to 38.7 and 21.2, respectively, on the eighth trial. *Mastigoproctus giganteus* also demonstrated the ability to learn a complex maze with six blind alleys. The percentage of subjects reaching the criterion (3 consecutive trials with fewer than 5 errors) in the complex maze increased from 5% on day 2 of training to 70% on day 14. *Mastigoproctus giganteus* also demonstrated the ability to learn to move from one compartment to another in a shuttle box to avoid a mild electric shock (avoidance learning). Additionally, results showed habituation of the threat display response after six repeated presentations of a non-aversive stimulus. This was the first demonstration of spatial reversal learning, complex maze learning, shuttle-box avoidance learning, and habituation in a whipscorpion. The suitability of *M. giganteus* as a model organism for studies on arachnid learning, as well as the adaptive significance of learning, are discussed.

Introduction

Learning is generally defined as a relatively permanent change in behaviour that occurs as the result of experience (Gormazano & Wasserman, 1990). Although the behaviour of arthropods has traditionally been associated with innate, closed behavioural programmes (Tinbergen, 1951), research conducted over the last decade has increased our awareness concerning the importance of learning and other forms of behavioural plasticity in the life histories of terrestrial arthropods (Papaj & Lewis, 1993; Menzel, 1999; Punzo, 2000a). Learning can enable animals to conserve energy by locating resources more efficiently. As a result, more energy may be allocated toward other activities such as reproduction and other physiological processes, nest construction, or defence of territory.

Research on learning allows investigators to address a number of relevant questions, including: (1) are interspecific differences in learning capacities related primarily to taxonomic status and/or differences in ecological niches? (2) under which specific conditions is information useful to an animal? (3) how does experience affect the decisions an animal must make in its search for resources? (4) how does learning in one species influence its interactions with conspecifics as well as other species? (5) which types of learning (e.g. habituation, classical conditioning, operant conditioning, association learning, spatial learning, complex learning/problem solving, etc.) are exhibited by various species? (6) what mecha-

nisms are responsible for the consolidation, storage and retrieval of experiential information in animal central nervous systems? (7) are specific features of the environment used as cues in the consolidation of information, and if so, which ones?

Most studies on arthropod learning have focused on insects (see reviews by Alloway, 1973; Papaj & Lewis, 1993; Punzo, 1996; Menzel *et al.*, 1997). With respect to arachnids, spiders (Araneae) have received most attention (LeGuelte, 1969; Lahue, 1973; Punzo, 2002a), with little or no information available on other arachnid groups (Foelix, 1996; Punzo, 2004). Some have argued that arachnids exhibit poor performance in learning studies and do not readily lend themselves to standard protocols used in the study of habituation, classical and operant conditioning, as well as association and spatial learning (Peckham & Peckham, 1887; Thorpe, 1963; Lahue, 1973).

More recent evidence, however, has indicated that this view has been overstated and is often not a function of diminished capacity, but rather a consequence of using tasks that are not well suited or ecologically relevant for spiders (Popson, 1999; Li & Lee, 2004). Spiders have demonstrated a capacity for learning in a number of different situations, in both laboratory and field experiments. For example, leg-position, shock-avoidance learning was demonstrated by the theraphosid spider *Aphonopelma chalcodes* Chamberlin (Punzo, 1988). *Phidippus regius* C. L. Koch (Salticidae) learned to avoid unpalatable prey as a result of previous foraging activities (Edwards & Jackson, 1994). Some lycosids and oxyopids can learn to associate chemical cues with the presence of prey (Punzo & Kukoyi, 1997) and potential predators (Punzo, 1997). The lycosids *Trochosa parthenus* (Chamberlin) and *Hogna carolinensis* (Walckenaer) learned to avoid an aversive stimulus in experiments using a T-maze (Punzo, 2002b) and complex maze (Punzo & Ludwig, 2002), respectively.

To my knowledge, no studies on the capacity for learning have been conducted on species within the order Uropygi (whipscorpions). The giant whipscorpion, *Mastigoproctus giganteus* (Lucas) (Thelyphoniidae), is a large (42–60 mm in body length for adults) and common component of the arachnid fauna of desert regions in the south-western United States and Mexico (Crawford & Cloudsley-Thompson, 1971; Punzo, 2001), and also occurs in scrub habitats in Florida (Muma, 1967). Males and females have a life span of 4–7 years and require 3–4 years to reach sexual maturity (Weygoldt, 1971; Haupt, 2000). There are 4 nymphal stages (proto-, deuto-, trito-, and tetranymph) and an adult stage (Weygoldt, 1971). This arachnid is typically nocturnal and wanders over the ground surface searching for suitable prey, mates, and shelter sites (Muma, 1967; Punzo, 2000b). *Mastigoproctus giganteus* usually exhibits a moderate to strong aversion to bright light (negative phototaxis) (Patten, 1917) and will quickly seek shelter within a crevice or under surface debris when exposed to sunlight in the field (Cloudsley-Thompson, 1968). When hunting, it moves slowly and explores objects that it encounters with its front legs,

which function as sensory structures (Crawford & Cloudsley-Thompson, 1971). When a suitable prey item is encountered, it is seized with the pedipalps. *Mastigoproctus giganteus* will excavate non-permanent burrows in moist sand or seek shelter in rock crevices, under surface vegetation, or in abandoned rodent burrows (Ahearn, 1970; Jackman, 1997). Because of its size, and its ability to survive and breed in captivity (Weygoldt, 1970; Punzo & Reeves, 2001), *M. giganteus* may lend itself as a suitable model organism for studies on the physiology and behaviour of Uropygi.

The purpose of this study was to investigate the learning capacity of *M. giganteus* with respect to several types of learning tasks: spatial learning in a T-maze and complex maze, avoidance learning (in a shuttle box), and habituation. These types of tasks were chosen for a number of reasons. Habituation is considered one of the simplest types of learning and is generally defined as the gradual fading of an unlearned response to repeated presentations of a non-aversive stimulus (Macintosh, 1974). Spatial learning tasks have ecological relevance for animals (like *M. giganteus*) that typically wander over variable distances searching for required resources (Davey, 1989; Papaj & Lewis, 1993; Punzo, 2004). Avoidance learning (AL) was chosen because there is a large body of literature on AL with respect to mammals (Bolles, 1975; Gormazano & Wasserman, 1990), but far fewer data available for arachnids (Punzo, 1996, 2004) and other invertebrates (Carew & Sahley, 1986). In addition, AL is a commonly used paradigm for studies on the effects of various pharmacological agents on vertebrate learning and memory processes that attempt to identify and explain molecular processes involved in consolidating and storing experiential information in animal central nervous systems (Macphail, 1982; White & Salinas, 1998). It is hoped that the results of this study will assess the suitability of *M. giganteus* as an arthropod model for studies on learning, both at the organismal level, as well as its potential for future studies addressing events at the molecular level.

Material and methods

Subjects

The whipscorpions used were offspring obtained from captive-bred animals that were originally collected at various locations in Big Bend National Park (Brewster County, Texas) and Las Cruces (Doña Ana County, New Mexico) during March and April 1999. The animals were maintained separately in plastic rodent cages (30 × 15 × 12 cm) and maintained at 60–65% relative humidity, 23 ± 0.2°C, and a 12L:12D photoperiod regime in Percival Model 85 environmental chambers (Boone, Iowa, USA). They were fed twice per week on a diet consisting of nymphs and adults of the cricket *Acheta domesticus*, adult beetles (*Carabus* spp.), yellow mealworms (*Tenebrio molitor*), and nymphs of the cockroach *Periplaneta americana*. First-instar nymphs were given fruitflies (*Drosophila melanogaster*) and first-instar cricket nymphs. All animals were provided with water *ad*

libitum. The number, age, and sex of animals used throughout these studies depended upon the availability of animals at the time that particular types of learning experiments were conducted. For all experiments, subjects (tritonymphs: 26–28 mm, 2.9–3.1 g; or adults: 43–44 mm, 7.5–8.5 g) were randomly selected from the offspring of parents collected from widely separated geographic locations, to ensure genetic heterogeneity among test subjects.

Spatial reversal learning using a T-maze

A standard vinyl T-maze designed for mice (Columbus Instruments, Columbus, Ohio, Model 0525M-D40) was used to assess spatial learning ability (Fig. 1). The walls of the entire maze were 12 cm in height and all runways (arms) were 9 cm in width. It contained a start box (SB: 12 cm in height, width and length) provided with a sliding panel that denied access to the main arm of the maze when closed. The main arm of the maze was 32 cm in length, and terminated in a “choice point” where the subject could turn at right angles into a right or left arm, each 20 cm in length. The floor at each end of these arms led to a goal box (12 × 12 × 9 cm). One of these boxes was transparent and allowed light to penetrate (S–), while the other was opaque (S+) and provided shelter from a light with an intensity of 1700 lux (aversive stimulus) that was suspended at a height of 15 cm over the centre of the maze.

All subjects (adult males; $n=5$) received 10 acquisition trials daily over an 8-day period (phase I), with an intertrial interval of 5 min. Pretraining consisted of 60 trials with both types of goal boxes accessible, during which individual spatial preferences were noted. In the following acquisition trials, individual non-preferred arms of the maze constituted a “correct” choice and led to the opaque goal box (S+). Animals were trained to an *a priori* criterion of 70% correct choices/day. Upon reaching the criterion in the acquisition trials (phase I), subjects were then exposed to 8 reversal trials (phase II) where they were required to choose the opposite arm in order to reach the opaque goal box (spatial reversal learning).

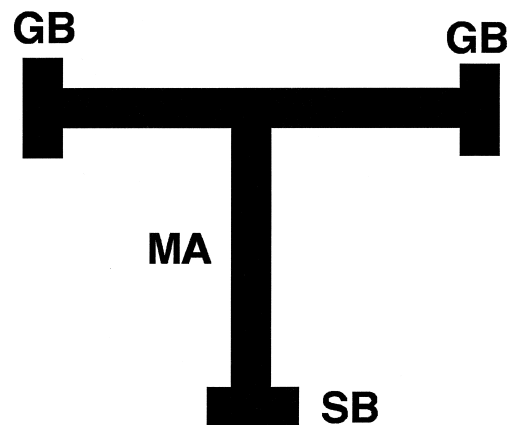


Fig. 1: Dorsal view of the T-maze used in these experiments. Maze consisted of a start box (SB) that led into the main arm (MA) of the maze, and on to a choice point between a right and left arm. All arms=9 cm in width; MA=32 cm in length.

At the start of each trial a whipscorpion was placed in the SB of the maze. The overhead light was turned on, which caused all subjects to move around the SB, touching the walls extensively with the front legs. After a period of 2 min, the sliding panel was lifted, allowing subjects to move into the main arm of the maze. If a subject made an incorrect choice and encountered the end wall of that arm (S –), it was allowed to correct its error. Data on the number of errors were recorded for each trial. After each trial, the walls and floor of the maze were cleaned using a damp soapy sponge followed by a 2% acetone solution in order to remove any residual odour cues from previous trials.

Data on the total number of errors and trials required to reach the criterion were recorded for subsequent analysis. All statistical procedures used throughout this study followed those described by Sokal & Rohlf (1995). A Bartlett's test showed homogeneity of variances, and G-tests indicated that the error variances were normally distributed: parametric statistical tests were used in data analyses (i.e. repeated-measures analysis of variance (ANOVA, *t*-tests).

Spatial learning in a complex maze

A diagrammatic representation of the complex maze used in these experiments is shown in Fig. 2. It had the same floor plan as the maze used by Turner (1913) in his work on learning in cockroaches, and has been used by numerous investigators over the years in studies on spatial learning in a variety of vertebrates and invertebrates (Gormazano & Wasserman, 1990). To summarise, it was constructed from galvanized iron, had a wooden floor, and a transparent glass cover. There were 5 blind alleys, a start box (SB), and an enclosed goal box (GB). A sliding wooden panel was fitted across the opening of the SB, preventing subjects from entering the main body of the maze until it was lifted. The same light source described above for T-maze experiments was

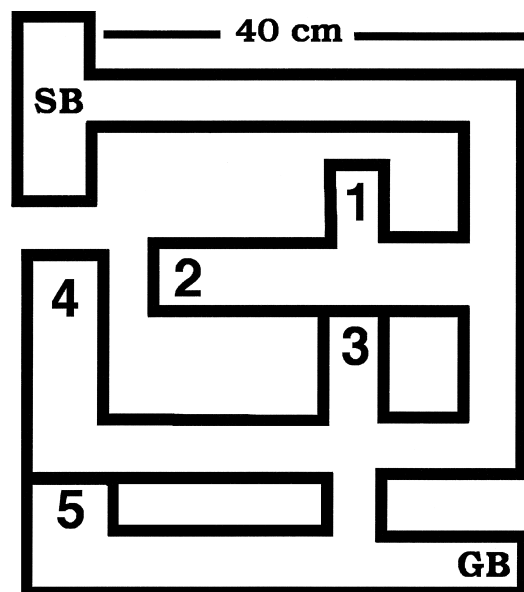


Fig. 2: Dorsal view of the floor plan for complex maze. Maze contained a start box (SB), goal box (GB), and 5 blind alleys.

used as an aversive stimulus to provide motivation for whipscorpions to traverse the maze and enter the opaque GB where they could find refuge from the aversive stimulus.

The protocol used in this study was similar to that reported by Punzo (2002a) for an analysis of spatial learning in the theraphosid spider *Aphonopelma hentzi* (Girard). To summarise, 20 adult male whipscorpions were used in these experiments. Each subject received 10 trials per day over a 14-day training period (acquisition phase). At the start of each trial, a subject was placed in the SB for a period of one min. The overhead light was turned on and the wooden panel was then lifted, allowing the whipscorpion to enter the main body of the maze. Subjects could avoid the bright light by correctly traversing the maze and entering the GB. Each subject was allowed to remain in the GB for 30 s, then retrieved, and placed back in the SB for 30 s before the next trial. After each trial the floor and walls of the maze were cleaned as described above for T-maze experiments. The number of blind alley errors and the time interval between leaving the SB and entering the GB (running time) were recorded for each trial. A subject was considered to make an error if more than half of its body entered a blind alley.

The criterion for learning was associated with the number of days required by subjects to complete 3 consecutive trials with fewer than 5 errors. Data were expressed as the percentage of subjects reaching the criterion on each day of testing. Twenty-four hr after reaching the criterion, each animal was subjected to 10 extinction trials/day for 6 days (extinction phase). During extinction, a transparent GB was used so that subjects were still exposed to the aversive light stimulus when they entered it.

Avoidance learning: shuttle box

The apparatus and protocol used in these experiments have been described in detail elsewhere (Cheney *et al.*, 1974). To summarise, the apparatus consisted of a wooden shuttle box (26 × 12 × 10 cm) divided by a sliding central partition into 2 opaque compartments of the same size (Fig. 3). The floor of each compartment was provided with a copper plate which completely covered its surface and through which a mild electric shock (50Hz, 16V, 3 sec) could be administered independently to each side. In any trial, the compartment receiving the electric shock was designated as the shock compartment (SC); the side receiving no shock was the escape compartment (EC).

Each subject was removed from its holding cage and placed in the shock compartment of the apparatus for 1 hr before testing. After this SC adaptation interval, the sliding door was raised and the amount of time required to enter EC (first latency, L1) was recorded with a stopwatch. The door was then lowered to prevent the subject from moving back to SC. A mild shock was then administered to the floor of the apparatus. After a 1-min interval, the door was raised again, allowing the subject to avoid the shock by moving back into the

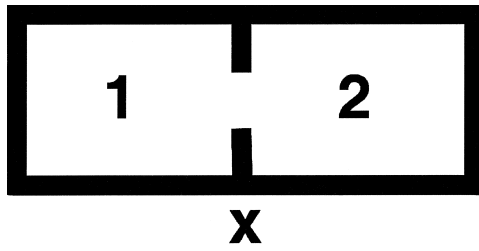


Fig. 3: Dorsal view of the shuttle box (SB) used in avoidance learning experiments. Floor of the apparatus was covered with a copper plate through which mild electric shock was administered. The SB consisted of two sections, a shock (1) and escape (2) compartment, and a partition between the two (x). SB was 26 cm in length, and 12 cm in width.

previous SC (now EC). If a subject failed to return, it was induced to move by gently prodding it with a glass stirring rod. The sliding door was lowered, and subjects were allowed to remain in the EC for a 1, 2, or 3-hr intertrial interval as described above. At the end of the intertrial interval, a shock was administered for one min to the compartment containing the whipscorpion. The door was raised and a second latency (L2) was calculated (the time interval required for the subject to enter the opposite compartment). Cut-off scores of 13 min (780 s) were used for both latencies (based on pilot studies which showed that none of the animals observed failed to shuttle back and forth between compartments within 13 min following the adaptation interval).

The difference between the first and second latency values for each subject was evaluated as percentage latency change (PLC), and was calculated according to the method described by Cheney *et al.* (1974): $(L2 - L1 / 780 - L1) \times 100$, when $L2 > L1$ (i.e. percentage of maximum possible increase in avoidance response), and $(L2 - L1 / L1) \times 100$, when $L2 < L1$ (i.e. percentage of maximum possible decrease). When $L2 = L1$, a zero value resulted.

One hundred and eighty tritonymphs (third-instar nymphs) were used for avoidance training, and were randomly assigned to 3 sets of 2 groups each ($n=30$ subjects/group): set 1: 1-hr experimental (1-HE) and 1-hr control (1-HC) groups, with 1-hr intertrial intervals; set 2: 2-hr intertrial interval experimental (2-HE) and control (2-HC) groups; and set 3: 3-hr intertrial interval experimental (3-HE) and control (3-HC) groups. Experimental groups received shock, and control groups received no shock.

Habituation experiments

All subjects used in these experiments were third-star tritonymphs. Nymphs and adults of *M. giganteus* exhibit a characteristic threat display when handled or disturbed by tactile stimuli. The display is also elicited by a puff of air delivered to the dorsal surface of the abdomen or cephalothorax (pers. obs.). The display consists of elevating the abdomen over a range of 20–90° to the ground and, depending on the perceived level of disturbance, this may be followed by the release of defensive

secretions from the pygidial glands (Schmidt *et al.*, 2000).

The test chamber consisted of a wooden enclosure (30 × 20 × 15 cm) with a floor covered with brown wrapping paper. A metal valve was fixed in place to a platform so that it stood at a height of 3 cm directly over the centre of the chamber. The valve was attached via rubber tubing to a cylinder of compressed air. The stimulus was a puff of air delivered for 1 s through the valve. Air was delivered at a flow rate of 20 cm/s in order to ensure that stimulus strength was constant for all trials.

Habituation was tested using 10 subjects. At the start of each trial a whipscorpion was transferred to the test chamber in a plastic container and placed on the centre of the floor, directly below the air valve. The air stimulus was presented over nine intervals (each interval=10 s) until the subject failed to respond for at least three consecutive presentations. Reactions of subjects were scored on a scale of 0 to 3, with 0=no response, 1=elevation of abdomen <45°, 2=elevation >50°, 3=a rapid movement of the subject away from its original position. For the tenth interval, following habituation to the puff of air, a novel stimulus was presented to each subject. The stimulus consisted of tapping the last segment of the pedipalps with a glass stirring rod. The presentation of a novel stimulus, following habituation to the initial non-aversive stimulus (air), was done in order to show that any diminution of the threat display was due to habituation and not fatigue.

Results

Spatial reversal and complex maze learning

Results for T-maze experiments are shown in Table 1. There was a significant decrease in the number of errors ($F=11.4$, $df=8, 32$, $p<0.05$) and trials ($F=8.99$, $df=8, 32$, $p<0.05$) to the criterion. Post hoc analyses of these effects using Duncan multiple range tests ($p<0.05$) showed improvement across reversals on both errors and trials to the criterion (the number of errors and

Phase	Number to criterion	
	Trials	Errors
Phase I (Acquisition)	127.7 (13.5)	92.3 (10.4)
Phase II (Reversals)		
1	107.3 (11.8)	75.6 (11.7)
2	116.5 (14.6)	71.3 (9.9)
3	95.8 (10.4)	62.5 (6.8)
4	86.2 (8.8)	55.7 (7.9)
5	63.9 (10.1)	42.8 (8.1)
6	48.3 (7.4)	33.6 (6.8)
7	40.6 (4.9)	35.4 (4.8)
8	38.7 (5.4)	21.2 (3.5)

Table 1: Number of trials and errors required by adult males of *Mastigoproctus giganteus* to reach the criterion for spatial reversal learning trials conducted in a T-maze. Upon reaching the criterion of 70% correct choices/day in acquisition trials (phase I), subjects were then exposed to 8 reversal trials (phase II). Data are expressed as means (\pm SD) for 5 subjects.

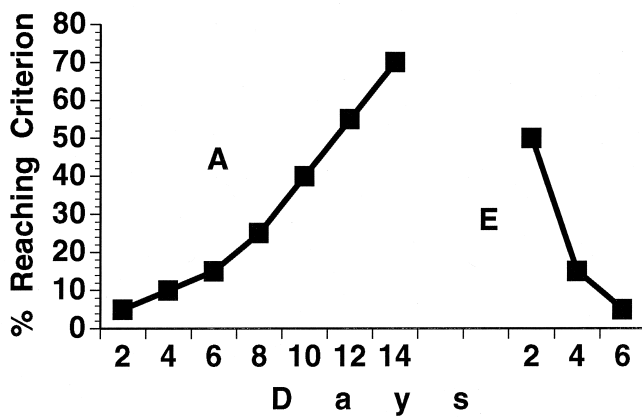


Fig. 4: Complex maze learning by adult males of *Mastigoproctus giganteus*. Data expressed as percentage of individuals ($n=20$) reaching the criterion for learning (3 consecutive trials with fewer than 5 blind alley errors). Each whipscorpion received 10 trials/day. Data shown for acquisition (A) and extinction (E) phases of the experiment.

trials was greater on the first reversal as compared with reversals 6 through 8).

Whipscorpions were also able to learn the complex maze (Fig. 4). A repeated measures ANOVA showed a significant effect of days of training on performance ($F=89.2$, $df=1$, 27 , $p<0.01$). Fifty-five percent of animals reached the criterion by day 12 of training, and 70% by day 14.

The number of blind alley errors decreased from a mean of 177.4 ± 18.8 SD on day 1 of the acquisition trials, to 23.3 ± 6.4 errors on day 14 ($t=19.7$, $p<0.01$). Running time also decreased, from a mean of $96.3 \text{ min} \pm 17.3$ on day 1 to $12.5 \text{ min} \pm 3.9$ on day 14 ($t=24.6$, $p<0.01$). Extinction was rapid, with only 15% of the subjects reaching the criterion by day 4 of the extinction trials (Fig. 4).

Subjects exhibited rapid walking movements in response to the aversive light stimulus in both types of mazes. They touched the walls of the mazes frequently with their front legs as they moved from the SB to the GB. In no case did a subject remain stationary in the SB and fail to enter the main body of the mazes, or remain stationary at the end of a blind alley without quickly turning around and continuing its exploratory movements.

Avoidance learning in a shuttle box

The results for the shuttle box experiments are shown in Table 2, and indicate that the whipscorpions were able to learn to shuttle between compartments to avoid electric shock. An ANOVA was performed on the data considering 2 factors: treatment (2 levels: experimental and control), and intertrial interval (3 levels: 1, 2 and 3 hr). Both factors caused a significant difference between levels (treatment: $F=38.44$, $df=1$, 174 , $p<0.01$; intertrial interval: $F=8.94$, $df=2$, 174 , $p<0.05$). There was no significant interaction between factors ($F=0.34$).

Habituation

The results for habituation are shown in Table 3. Habituation was demonstrated by these whipscorpions because all subjects stopped responding to the puff of air by the seventh interval. This decremental responsiveness of these subjects from the first to the ninth interval was significant ($F=18.24$, $p<0.01$). Following habituation, when subjects were presented with a second non-aversive stimulus (tapping of the pedipalps during the tenth interval), they again exhibited the threat display, suggesting that the waning of the original response to air was due to habituation and not general fatigue.

Discussion

These results show that *M. giganteus* demonstrated reversal learning in a T-maze, the ability to learn a complex maze, avoidance learning in a shuttle box, and habituation (threat display). To my knowledge, this is the first demonstration of learning capacity for a whipscorpion.

Because of the strong negative phototaxis characteristic of *M. giganteus*, bright light is an effective aversive stimulus that will serve as a motivational factor for maze learning studies in this species. *Mastigoproctus giganteus* demonstrated the ability to learn a reversal task in a T-maze. Habit reversal has been studied extensively in mammals but far fewer data are available for invertebrates (Bitterman, 1975; Able, 1991). Animals are often confronted with unpredictable or changing conditions in the quality or quantity of food and other resources. Such conditions can change over the course of days, weeks, or months. Animals that can learn to switch (habit reversal) to food items that may have been avoided when more attractive foods were in greater abundance may have an advantage over those who lack this ability (Benhamou & Poucet, 1996). If a normal escape route becomes inaccessible, animals that can learn to reverse their path and seek an alternative route to another shelter site will improve their chances for survival.

The results for complex maze learning are also significant with respect to survival. It has been suggested that spatial learning ability is a necessary prerequisite for most animals to survive in their natural habitats (Holtzman, 1998). The ability of an animal like *M. giganteus*, that typically moves over variable distances to obtain

Set	Percentage latency	
	Experimental group	Control group
1	55.3	+1.9
2	49.8	-6.2
3	24.4	-18.3

Table 2: Percentage latency change (PLC) as a function of intertrial interval for *Mastigoproctus giganteus* in avoidance learning experiments. Set 1: 1-hr intertrial interval for experimental (1-HE) and control (1-HC) groups; set 2: 2-hr intertrial interval for experimental (2-HE) and control (2-HC) groups; set 3: 3-HE and 3-HC, respectively. Experimental groups received mild electric shock; controls did not.

Subject	Response score at interval									
	1	2	3	4	5	6	7	8	9	10
1	3	3	2	2	1	2	0	0	0	2
2	2	2	1	0	1	1	0	0	0	1
3	2	1	1	0	1	1	0	0	0	2
4	3	2	0	1	1	1	0	0	0	3
5	2	1	2	1	1	0	0	0	0	3
6	2	2	1	1	1	2	0	0	0	1
7	3	1	1	0	2	0	0	0	0	2
8	2	2	1	2	1	1	0	0	0	2
9	2	1	2	2	0	1	0	0	0	2
10	1	2	1	1	1	0	0	0	0	3

Table 3: Habituation response scores of 10 nymphs of *Mastigoproctus giganteus* to a tactile stimulus (puff of air) presented at nine 10-s intervals. Following habituation to the initial non-aversive stimulus, a novel stimulus (tapping cephalothorax with a glass rod) was presented during the tenth interval. Response scores: 0=no response; 1=elevation of abdomen <45°; 2=elevation of abdomen >50°; 3=rapid movement away from the stimulus.

resources, to learn more efficient foraging or escape routes will have beneficial consequences. It will reduce the time spent in random search patterns, thereby decreasing energy expenditure and potential exposure to predators. The ability to associate a specific location with the availability of food, shelter sites or escape routes can also enhance overall fitness.

Avoidance learning is a type of operant conditioning (Davey, 1989). In avoidance learning protocols, the animal is typically required to move in order to avoid some noxious consequence. A common approach has been the use of an apparatus known as a shuttle box which contains two compartments. The subject is required to move between the compartments in order to avoid mild electrical shock. In this study, *M. giganteus* demonstrated the capacity for avoidance learning in a similar apparatus. This type of protocol has been used extensively by researchers interested in analysing the effects of pharmacological agents on learning and memory processes in mammals as well as identifying neurochemical events associated with consolidation of experiential information in the central nervous system (Cheney *et al.*, 1974; Bolles, 1975; Punzo, 1996; White & Salinas, 1998). The results of this study suggest that *M. giganteus* may be a suitable organism for similar studies using invertebrate models.

The capacity for avoidance learning also allows animals from numerous vertebrate and invertebrate taxa to learn to avoid food items that contain noxious or toxic chemicals by associating their ingestion with unpleasant consequences (see reviews by Chivers & Smith, 1998; Kats & Dill, 1998). With respect to arachnids, for example, the araneid spider *Araneus diadematus* Clerck will learn to avoid prey items that have been treated with repellent chemicals (Bays, 1962). Punzo (1997) showed that once wolf spiders (*Schizocosa avida* (Walckenaer)) had an encounter experience with a naturally-occurring scorpion predator (*Centruroides vittatus*) and were able to escape, they would subsequently avoid substrates containing odour cues associated with this scorpion. Conversely, no similar aversion was exhibited by spiders that had never encountered a scorpion. Thus, animals learn to avoid eating toxic or unpalatable foods and to

avoid potential predators, thereby increasing their survival capacity.

The threat display appears to be a reliable behavioural act for the study of habituation in *M. giganteus*. Habituation is often described as one of the simplest forms of learning and has been observed in a wide variety of taxa including protozoans, cnidarians, annelids, molluscs, arthropods, and vertebrates (Maier & Schneirla, 1933; Thorpe, 1963; Gormazano & Wasserman, 1990). The initial novel stimulus is non-aversive, and the response it elicits is one usually associated with danger such as fleeing, crouching, startle responses, or defensive displays. After repeated occurrence without significant effect the stimulus loses its novelty and is ignored. The adaptive significance of habituation can be explained in terms of energy budgets. Animals will conserve energy by learning to ignore stimuli that have no negative consequences, and as a result will be able to allocate their energy reserves toward other activities such as foraging, reproduction, mating, and territorial defence.

In conclusion, the performance of *M. giganteus* on a number of learning tasks indicates that this arachnid can serve as a valuable subject for future studies on the behavioural or biochemical aspects of invertebrate learning processes.

Acknowledgements

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