

Alterations in Habituation of the Tail Flip Response in Epigeal and Troglotic Crayfish

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ABSTRACT We demonstrate that the probability of the crayfish, *P. clarkii*, to tail flip in response to a touch on the dorsal tail fan is dependent on both the size and the behavioral state of the animal. Alterations in the animal's internal physical state, such as when the animal autotomizes its chelipeds, will cause larger-sized animals to tail flip; if they were not autotomized, then no tail flip response would occur. Altering the external environment by removal of water causes small crayfish, which normally habituate slowly, to rapidly habituate. Observation of large adult crayfish in a species, *O. australis packardii*, one that evolved to live in total cave darkness, revealed that they are more likely to tail flip than are the sighted, adult *P. clarkii*. Results indicate that the behavioral state of the crayfish can result in rapid and long-term alterations in the tail flip response and in habituation rates to repetitive stimuli. This ability to show plasticity in gain setting may be regulated by neuromodulators and can occur in large adults of the sighted crayfish. Differences between the two species indicate that size may not be the sole contributing factor to account for tail flip behaviors. *J. Exp. Zool.* 290:163–176, 2001. © 2001 Wiley-Liss, Inc.

Prey use several tactics in order to evade predators. These include hiding, being motionless, or rapidly escaping. For instance, crayfish have developed the tail flip to escape the predation of birds, fish, reptiles, and other crayfish (Krasne et al., '97; Edwards et al., '99). Different responses are utilized for different threats (Wilson, '75) and these responses may change as the animal grows (Lang et al., '77; Holekamp and Smale, '93; Pavey and Fielder, '96). How animals respond to various sensory stimuli during different developmental stages of their life depends on many factors (Dimarco and Hanlon, '97; Neat et al., '98). For example, smaller crayfish are more likely to tail flip during an encounter with a larger crayfish (Pavey and Fielder, '96), and experience plays a role in determining this behavior (Copp, '86). Also, responses to various stimuli depend on the sensing acuity of an animal. Much of our understanding of sensory systems is derived from examining individual sensory systems isolated from the organism as a whole (Atema et al., '88). Approaching the situation in this manner does not accomplish the ultimate goal of understanding the animal's behavior (Atema et al., '88; Burmistrov and Shuranova, '96; Zulantz-Schneider et al., '99).

In order for the animal to survive, the animal's behavioral response should be influenced by a variety of selection pressures. For instance, a crayfish must balance the need to escape with a

massive tail flip, which utilizes energy, before knowing the cause of a stimulus on its tail. It is known that the crayfish social structure impacts the willingness of the animal to tail flip to a given stimulus (Bruski and Dunham, '87; Guiasu and Dunham, '97). Consequently, in promoting the survival of the species, plasticity of the nervous system may play an important role in allowing the animal to rapidly adapt to threatening stimuli.

Additionally, animals evolve to fit their particular environment by making use of sensory information to favor reproduction and survival (Enquist and Leimar, '83; Dukas, '98; Elwood et al., '98). Over time, this adaptation can result in altered anatomical and physiological abilities (Culver et al., '95). Comparing the modulation of the escape behavior in sighted crayfish to the escape behavior of a species with different anatomical and physiological abilities provides information on how a different sensory system affects behavior.

The evolutionary changes observed in blind cave crayfish suggest the importance of nervous sys-

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tem plasticity in promoting the survival of the species. In addition to providing a good comparison for blind cave crayfish, sighted crayfish make a good model for further exploring the sensory system involved in the tail flip response. In most animals, the dissection of individual neurons and neural circuits to understand the complex integration of sensory and motor systems is extremely challenging. Crayfish serve well as a model organism for investigating escape behaviors. They have evolved particular behaviors that can be quantified and investigated even at the level of neural circuitry. For example, the tail flip response in relation to the age of the animal and the size of the neurons with the circuit has been examined (Fricke, '86). Furthermore, the sensory difference in blind cave crayfish (*Orconectes australis packardii*) suggests that they might have evolved a different responsiveness to tail flipping in relation to the developmental stages. It seems reasonable that the blind cave crayfish do not have the advantage of responding to visual stimuli through various neuronal circuits to elicit a tail flip as do sighted crayfish. On the other hand, it is assumed that the blind cave crayfish rely heavily on their other senses to meet the challenges of cave life in total darkness (Barr and Holsinger, '85; Culver et al., '95). These unique adaptations may show differences in the habituation of the crayfish due to changes in the animal's sensory state.

Comparing the blind cave crayfish responses with previous studies on sighted crayfish provides insight into the factors that drive selection for the tail flipping behavior. Previous studies dealt with the tail flip response being advantageous to the animal at various stages of its life. Juvenile, sighted crayfish are more likely to respond to threatening stimuli by tail flipping, while adult crayfish are more likely to respond with the defensive use of their chelipeds. Fricke ('86) noted that the chelipeds of the juvenile crayfish account for little of the animal's total body mass when compared to that of an adult. Thus, juveniles can tail flip without the energy cost of moving the large mass of the cheliped. Morphologically, the thinner exoskeleton of small crayfish makes them less able to withstand the stress of predation. Consequently, they may have evolved greater tail flipping capabilities. Mature adults of *Procambarus clarkii* will not exhibit tail flips when both of their chelipeds are intact, and it has been suggested that the disproportionate development of cheliped mass to body mass of the animals does not favor

tail flipping in adults (Fricke, '86). We have shown that *O. a. packardii* exhibit even a greater ratio of cheliped length to body length. Yet, a much higher occurrence of tail flipping exists in adult cave crayfish than for adults of *P. clarkii*. Conclusively, tail flipping behavioral differences among species of crayfish are not just a function of body proportions. The blind cave crayfish's sensory state may be an additional factor in generating tail flipping behavior.

Another facet, the neural circuitry of the tail flip response, has been examined extensively (Krasne and Wine, '75; Olsen et al., '96; Krasne et al., '97; Yeh et al., '97). Krasne and Wine ('75) identified three pathways crayfish use to elicit a tail flip response: lateral giant, medial giant, and nongiant. Krasne and Wine ('75) used touches on the tail in order to elicit lateral giant responses. They initiated further studies of the neural circuitry by eliciting lateral giant responses to drive the tail flip behavior. Subsequently, Fricke ('86) noted that stimuli given every 30 sec caused intermediate-sized (<10 cm) crayfish to show habituation. The same protocol used on smaller individuals elicited habituation resistant behavior. Later, a physiological explanation was proposed by Edwards et al. ('94). As a crayfish grows, there is an increasing predominance of the depression-prone synapses in carrying information from the sensory neurons. These results demonstrate that larger animals have a faster rate of habituation than do smaller ones.

Recently, modulation by serotonin (5-HT) of the sensory induced tail flip response has been observed among socially dominant and submissive crayfish (Yeh et al., '96; Krasne et al., '97). These observations suggest a hormonal influence on the neural circuitry. The development of social status requires less time than that needed to grow from a small crayfish to a large crayfish, but it still takes from several minutes to days of interaction time for the establishment of a long-term social structure. Earlier studies did not address a minimal time required for a dominant or submissive animal to show differences in sensitivity to the exogenous application of 5-HT on the semi-intact preparations. A link has been established between claw removal and the tendency to increase tail flips generated by medial and nongiant pathways (Krasne and Wine, '75; Lang et al., '77). These previous studies have examined modulation of the crayfish tail flip response over long periods of time; however, they have not examined the habituation of the tail flip response for pos-

sible modulatory influences over a short term basis (i.e., seconds). It seems reasonable that the survival of the crayfish would be aided by the ability to modulate the tail flip response. Change within short periods of time and to a variety of factors would promote crayfish survival. The tail flip response could be modulated from direct neural input within the circuitry and/or by release of neuromodulators from "gain setting" neurons (Ma et al., '92; Hörner et al., '97).

Portions of this data have been presented previously in abstract form (Kellie et al., '99).

MATERIALS AND METHODS

Crayfish care

Sighted *Procambarius clarkii* were kept in isolated tanks within the lab. Crayfish were kept in isolation to avoid hormonal changes associated with establishing social dominance (Huber et al., '97). The crayfish were fed dry fish food and kept in a light:dark cycle of 16:8 hr. Dark-adapted crayfish were kept in 24-hr darkness. The blind cave crayfish, *Orconectes australis packardii*, were obtained from the Sloan's Valley Cave System (Burnside, KY). The cave crayfish were transported to the Lexington laboratory in dark, chilled (18°C) water. At least 1 week elapsed before the cave crayfish were tested. They were stored in isolation and in total darkness at cave-like temperatures (20°C).

Testing procedure

Testing followed a behavioral approach similar to methods used by Krasne and Wine ('75). Crayfish were tested in an approximately 30.5 cm × 30.5cm × 15.24 cm tank. Crayfish movement about the tank was unrestricted. The experiments were performed in the presence of a dim red light (Edmond Scientific, filter model # D43,951, using a 40-W incandescent bulb hanging at a distance of 2 m above the testing tank). The examiner remained still and to the side of the overhead dim red lighting of the crayfish in order not to induce a visual response. After each crayfish was tested, the water in the tank was replenished. During the experiment, the crayfish received a touch on its tail once every 30 sec. Tests continued until the subject exhibited a significant number of consecutive failures to tail flip. An infrared-sensitive video camera recorded these results.

Five different experimental groups were observed to determine if a change in the habituation rate was present for tail flip responses. These

groups were as follows: (1) Group 1 consisted of tests on sighted crayfish of various sizes (small, intermediate, and large); (2) Group 2 consisted of sighted small and large crayfish with a single cheliped and/or both chelipeds removed; (3) Group 3 consisted of juvenile sighted crayfish in a tank with moist substrate; (4) Group 4 consisted of sighted large and small crayfish kept in the dark for a period of 2 weeks prior to testing; and (5) Group 5 consisted of large cave crayfish maintained in cave-like conditions.

An independent series of tests was performed on the cave crayfish to judge their social interactions. After being isolated for 2 weeks, a group of six cave crayfish were placed together all at once. This experiment examined variance in the number of social interactions with time. Also, the fluctuation of tail flipping during crayfish interaction over time was examined. Monitoring of the animals occurred with an infrared-sensitive camera. During these studies, the only illumination came from an infrared light. Initially, this study was conducted in a large tank. The tank diameter was gradually reduced. Three sizes of circular environments were used (large: 86 cm diameter and 0.581 m²; medium: 45.6 cm diameter and 0.163 m²; smallest: 22.8 cm diameter and 0.041 m²). Each setting was maintained for 48 hr. In some cases, an experiment was stopped if some individuals dug underneath the wall. The walls consisted of garden-siding foil. The substrate was a mixture of sand and fine gravel mixed with dirt. This allowed the crayfish to make depressions, not burrows, within the substrate. This was designed to be as close as possible to the conditions in the region of the cave from which these particular crayfish were obtained.

Descriptive data for each sighted and blind crayfish used were recorded and are presented in Table 1.

RESULTS

To examine habituation rates in the tail flip response based on size of *P. clarkii*, three distinct size groups were tested (Fig. 1A–C). The group of large individuals consisted of the largest adults commonly found in the field (Raceland, LA). The size of small crayfish compares to earlier reports on habituation of tail flip response (Fricke, '86). Small crayfish were the same size as the adult cave crayfish used in this study (Fig. 1D, Table 1). The intermediate-sized group of *P. clarkii* provided a third grouping to determine a size relationship in the habituation response.

TABLE 1. Morphological characteristics of epigean and troglobitic crayfish

	Length						Mass		
	Abdomen (cm)	Thoracic (cm)	Cheliped		Propus		Total (grams)	Cheliped	
			(cm) L	(cm) R	(cm) L	(cm) R		(grams) (ml) R (volume)	(grams) (ml) L (volume)
<i>P. clarkii</i>	4.4	5.0	5.6	5.6	1.0	1.0			
	3.4	3.6	3.7	3.7	0.8	0.7			
	3.2	3.2	3.8	3.8	0.7	0.6			
	3.5	4.2	5.6	5.4	0.9	0.9			
	5.0	4.5	8.5	8.9	2.0	1.9			
	5.1	5.5	8.7	8.7	1.9	1.8			
	5.2	5.1	7.9	7.0	1.8	1.5			
	2.6	2.5	2.3	2.5	0.5	0.5			
	3.1	3.3	4.0	4.0	0.8	0.8			
	3.0	3.1	2.9	3.0	0.5	0.5			
	4.5	4.5	7.5	7.0	1.6	1.5			
	5.0	5.3	6.6	6.6	1.2	1.2			
	5.0	5.4	7.5	7.4	1.6	1.5			
	5.0	5.0	8.0	8.0	1.7	1.7			
	5.5	5.5	6.9	6.5	1.4	1.4			
	3.6	3.5	—	3.7	—	0.7			
	3.5	4.0	3.2	3.2	0.6	0.6			
	4.0	4.3	6.9	7.0	1.5	1.5	18.00		
	4.2	3.7	7.1	7.1	1.5	1.5	18.55		
	4.0	4.2	7.0	7.2	1.6	1.4	19.95		
	4.0	3.7	6.7	6.9	1.3	1.3	18.33	2.56 (2.0)	2.57 (2.0)
	3.9	3.9	5.5	5.5	1.2	1.2	14.09	1.38 (1.0)	1.38 (1.0)
	3.9	4.0	5.0	4.9	0.8	0.8	14.20	0.89 (1.0)	0.91 (0.5)
	4.5	4.3	6.5	7.1	1.2	1.5	20.70		
	4.1	4.0	6.0	5.9	1.2	1.3	15.81	1.63 (2.0)	1.66 (1.0)
	3.2	3.2	5.6	5.7	1.0	1.0	13.20	0.96 (1.0)	0.93 (1.0)
	3.5	3.5	5.1	5.1	1.0	1.0	10.20		
	3.6	3.5	4.0	4.2	0.7	0.7	9.10	0.51 (0.4)	0.33 (0.3)
	3.0	3.2	3.8	3.8	0.6	0.6	7.50		
	3.2	3.5	4.0	4.0	0.6	0.7	8.50		
	3.5	3.5	4.5	4.5	0.8	0.8	9.40		
	3.1	3.2	3.5	0.4	3.5	0.4	6.80		
	3.5	3.3	4.1	4.1	0.5	0.5	8.55		
	3.4	3.5	4.2	4.2	0.5	0.6	8.80		
	3.3	3.4	3.7	3.8	0.7	0.8	7.19		
	2.9	3.0	3.9	3.9	0.7	0.7	6.28		
	3.3	3.1	3.7	3.7	0.5	0.6	7.39		
	3.4	3.0	3.4	3.4	0.5	0.4	6.95		
	3.0	3.0	3.7	3.7	0.4	0.5	5.80	0.41 (0.3)	0.42 (0.3)
	3.3	3.0	4.0	0.4	0.5	0.5	7.70		
	3.5	3.7	4.3	4.3	0.6	0.7	8.59		
	3.4	3.3	3.8	3.9	0.6	0.6	7.50		
	2.9	2.9	3.1	3.1	0.4	0.4	4.45	0.44 (0.15)	0.14 (0.15)
	3.2	3.3	3.5	3.5	0.5	0.4	7.20		
	3.0	3.2	3.7	3.7	0.5	0.5	7.10		
	3.3	3.2	3.7	3.7	0.6	0.6	7.56		
	3.5	3.3	4.0	4.0	0.7	0.7	8.70		
	3.1	3.1	3.8	3.8	0.5	0.6	7.03		
	3.6	3.7	4.0	4.2	0.6	0.7	8.61		

(Continued)

In order to index the tail flip response, a score of one was given if the animal tail flipped. A score of zero was given if the animal failed to tail flip or merely moved away. This index standardized

the responses among the three differently sized groupings. A representative trail for an individual within each of the size categories is shown in Fig. 2A. In order to better describe the behavior of a

TABLE 1. (Continued).

	Length						Mass	
	Abdomen (cm)	Thoracic (cm)	Cheliped		Propus		Total (grams)	Cheliped (volume) (grams) (ml)
			(cm) R	(cm) L	(cm) R	(cm) L		
<i>O.a. packardii</i>	3.1	2.2	3.6	3.5	0.6	0.6	3.55	
	2.2	1.9	2.8	2.8	0.5	0.5	1.29	
	2.8	2.3	3.2	3.1	0.5	0.5	2.18	
	2.9	2.7	4.0	3.8	0.7	0.7	3.47	
	2.5	2.2	3.3	3.1	0.4	0.5	2.00	
	2.2	1.9	2.7	2.7	0.5	0.3	1.38	.082 (0.10)
	2.8	2.9	3.2	3.1	0.6	0.6	2.56	
	2.1	2.0	3.0	2.5	0.5	0.3	1.75	
	2.8	2.1	3.1	3.1	0.6	0.6	2.42	
	2.5	2.3	3.6	3.7	0.7	0.7	2.55	.193 (0.15)
	2.6	2.2	3.3	3.3	0.6	0.6	2.40	.182 (0.10)
	2.6	2.2	3.2	3.0	0.7	0.6	2.30	.180 (0.10)
	2.8	2.1	3.6	3.6	0.7	0.7	2.48	
	2.1	2.0	3.0	2.8	0.5	0.5	1.46	.082 (0.10)
	2.5	1.8	3.3	3.1	0.5	0.5	1.72	
	2.0	1.7	2.4	2.5	0.4	0.5	1.14	
	2.0	1.6	2.0	2.0	0.3	0.4	0.92	
	2.7	2.2	3.5	2.9	0.7	0.6	2.37	
	1.8	1.6	1.9	1.8	0.3	0.3	0.72	.041 (0.07)
	2.2	2.0	3.3	2.9	0.6	0.4	2.13	
	1.6	1.8	1.8	1.8	0.3	0.3	0.54	.022 (0.01)
	2.0	1.7	2.3	2.3	0.4	0.3	0.98	
	2.7	2.2	3.1	3.0	0.5	0.5	2.26	
	2.6	1.7	2.7	2.5	0.4	0.4	1.14	
	2.6	2.3	4.1	4.0	0.8	0.8	2.63	
	2.8	2.4	4.2	4.0	0.9	0.8	3.13	

group as a whole, the number of crayfish responding at a particular time was divided by the total number of crayfish tested within that size group. This provided a percent response for the combined six animals for the small- and intermediate-sized groups (Fig. 2B). Since the large animals did not produce tail flips the combined response is not shown. The percent of tail flips is significantly greater for each time point from the onset to 45 min for the small sized animals as compared to the intermediate sized group ($P < 0.05$, nonparametric, Wilcoxon rank sum test).

By adding the fractions of the number of crayfish that tail flipped per total number of crayfish tested for each time period, a cumulative sum with respect to time was obtained. Graphically, this provides an easy comparison for a total number of tail flips for each group of sized crayfish over time (Fig. 3). It is clearly evident that the smaller crayfish tail flipped more over time than either the intermediate and large crayfish. It is also apparent that the intermediate-sized crayfish habituate sooner (~30 min) than the smaller crayfish. The large crayfish did not exhibit any tail flip-

ping behavior. By this type of analysis, one can compare influences of the environmental, intrinsic state, and inherent species differences on habituation patterns.

To examine if an altered internal state of the animal leads to an escape behavior when a crayfish has a reduced potential for defending itself to a potential predator's attack from behind the animal, the chelipeds were removed and stimuli were given to the telson while monitoring the animal's tail flip behavior. Ten intermediate sized *P. clarkii* were tested under the standardized conditions and the group response is provided in Figure 4A. Once a crayfish showed 10 repetitive failures it was alternatively divided into one of the following two groups: sham or clawless. In all, five of the initial individuals had both chelipeds rapidly removed by forcefully pinching at the base of the chelipeds to induce autotomization. The other five individuals, the sham group, were handled in the same manner except for the forceful pinching. Distinct differences in the subsequent testing for the tail flip response between the two groups are readily apparent (Fig. 4B₁ and



Fig. 1. Three various sizes of the sighted *Procambarus clarkii* and adults of the blind cave crayfish, *Orconectes australis packardii* were examined for habituation of the tail flip response. The large *P. clarkii* is a mature adult (A), where as the medium-sized is intermediate (B) in comparison to small

or large. The small *P. clarkii* (C) are close in size to the adult *O. a. packardii* (D). Note the ratio of cheliped length to body length for *O. a. packardii* is greater than for small *P. clarkii*. Scale: 10 cm.

B₂). Retesting after one week of isolation showed the differences still existed between the two groups (Fig. 4C₁ and C₂). The percent of tail flips is significantly greater for comparisons of averaged 5-min time periods from the onset to 40 min for the clawless animals as compared to the sham group for both the one and two week trials ($P < 0.05$, nonparametric, Wilcoxon rank sum test, $n = 5$). The differences are more apparent in the cumulative plots between the different groups (Fig. 5A and 5B). The group in which the claws were removed remained more resistant to the habituation than the sham group. It is also apparent that handling the crayfish in a subsequent testing resulted in an increased initial excitability. This can be seen in the recovery from depression by the sham control animals in a short period of time (Compare Figs. 4A and 4B₂ sham).

In order to avoid repetitive testing of the same individuals, pristine experimental groups were

tested with their chelipeds intact or removed for both large ($n = 6$) and small ($n = 6$) crayfish. Another group of large crayfish was tested with only one cheliped removed ($n = 5$). In the small *P. clarkii*, presence or absence of chelipeds did not produce any large shifts in the total number of tail flips. They remained quite responsive to the stimulus on the tail. Likewise, the rate of habituation remained similar between the two groups (Fig. 6A). Compared to large crayfish with two chelipeds intact, large crayfish demonstrated a pronounced alteration in behavior with one or both chelipeds removed (Fig. 6B). The fact that large animals rapidly exhibit changes in the tail flip response suggests that the synaptic circuitry and/or efficacy are not constant but can be rapidly modulated within a few minutes. Thus, the exogenous stress, or hormones released during limb autotomy, lead to alteration in the intrinsic state of the crayfish. This alteration changes the rela-

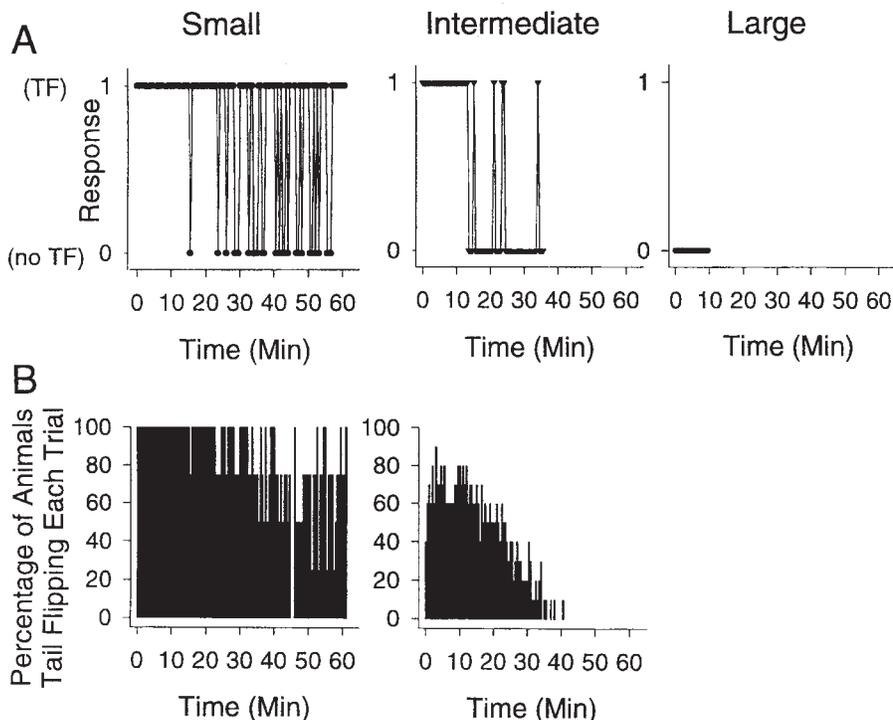


Fig. 2. Differences in habituation rates are observed among the three size classes of *P. clarkii*. A representative individual is shown for its habituation rate to tail flip for a stimulus given every 30 sec (A). If the animal tail flipped, it was given a score of one. A failure to tail flip is represented by a zero. Experiments ended after the crayfish failed to tail flip 10 consecutive times or 1 hr had elapsed. Compiled responses

in the percent of crayfish that tail flipped for a group of 10 individuals of both small and intermediate sizes are shown in (B). The percent of tail flips is significantly greater for each time point from the onset to 45 min for the small-sized animals as compared to the intermediate-sized group ($P < 0.05$, nonparametric, Wilcoxon rank sum test).

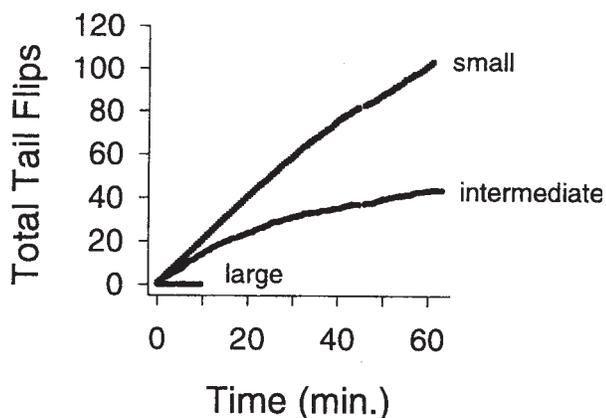


Fig. 3. Comparisons in the rates of habituation of tail flips among the three sizes of *P. clarkii* are represented in the cumulative profiles. The cumulative profiles represent the total number of tail flips from all crayfish in a group up to and including a particular time point. The rate of rise for a line indicates the rate at which animals within that group are continuing to tail flip over time. When a plateau is reached, it indicates that tail flipping has discontinued. Note the group of large crayfish did not tail flip. Thus, the response remained at zero. The group of small crayfish tail flipped for the longest amount of time before habituating.

tively static response of not tail flipping to a response favoring tail flipping among the large crayfish. Perhaps no changes are observed in the small crayfish data, because their normal response is to tail flip, even with their chelipeds intact.

In an attempt to examine other factors that might alter the state of excitability, large *P. clarkii* were maintained in complete darkness for 1 week before testing tail flip responsiveness. We hypothesized that these large animals, which normally do not tail flip, might be sensitized to tactile stimuli and be more excitable as a consequence of being maintained in the dark. On the contrary, the animals did not show any differences that we could observe under the stimulation paradigm used (data not shown). However, dark-adapted, small, sighted crayfish habituated sooner than those kept in normal lighting, as demonstrated in cumulative plot (Fig. 7A).

When the environment was altered by water removal from their tank, there was a large reduction in the total number of tail flips and the rate of habituation for small crayfish (Fig. 7B). The

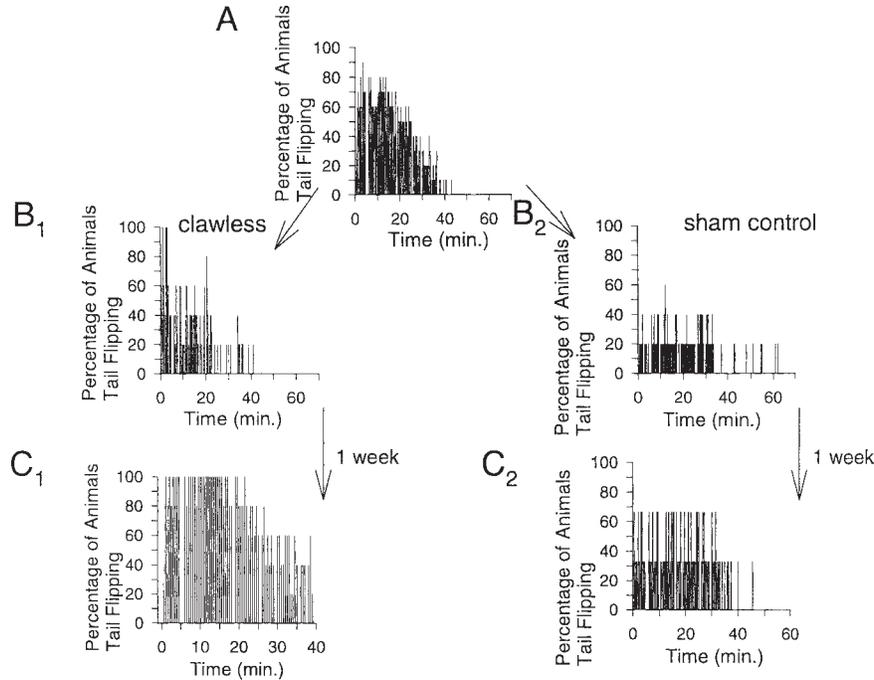


Fig. 4. The rapid effects of claw removal on habituation rates in intermediate sized crayfish demonstrate the neural plasticity of the circuit. Ten crayfish were tested (A). Immediately following the test, five of the crayfish had their chelipeds removed and were re-tested (B₁). The other five crayfish were held as if their chelipeds were removed (B₂). One week later, the same groups were re-examined (C₁ and C₂). The

percent of tail flips is significantly greater for comparisons of averaged 5-min time periods from the onset to 40 min for the clawless animals as compared to the sham group for both the initial and 1-week trials (for averages of 5-min periods in each distribution $P < 0.05$, nonparametric, Wilcoxon rank sum test, $n = 5$).

small animals were used, because they demonstrated the highest probability of tail flipping behavior when submerged in water. It is interesting that the small crayfish continued tail flipping even out of water. Tail flipping may serve as an escape tactic even when crayfish leave pools of water.

Some species of crayfish may be expected to be more excitable and show less habituation to such stimuli on the telson. Thus, the unsighted cave

crayfish, *O. australis packardii*, were observed and compared to the same-sized individuals of the sighted *P. clarkii* (small). A complication exists in that the fully mature cave crayfish are the same size of the small *P. clarkii* used in this study. Thus, the body proportions of cheliped mass to body mass may not be comparable between the small *P. clarkii* and the adult cave crayfish.

In keeping with earlier studies (Fricke, '86) re-

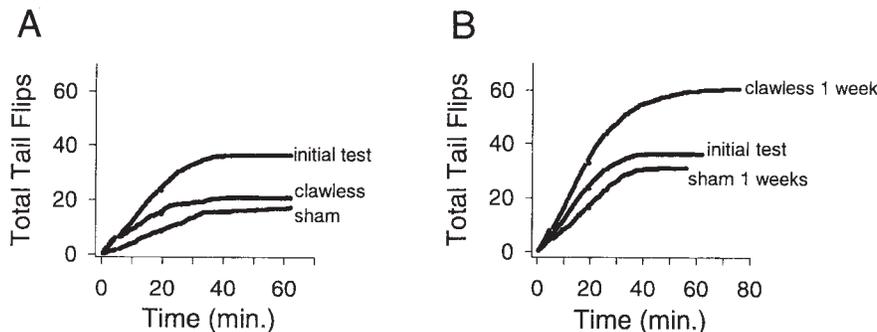


Fig. 5. Cumulative sum in the numbers of animals continuing to tail flip over time indicate that the crayfish with both chelipeds removed had a reduced habituation as com-

pared to the sham controls for the initial claw removal period (A) and following one week afterwards (B).

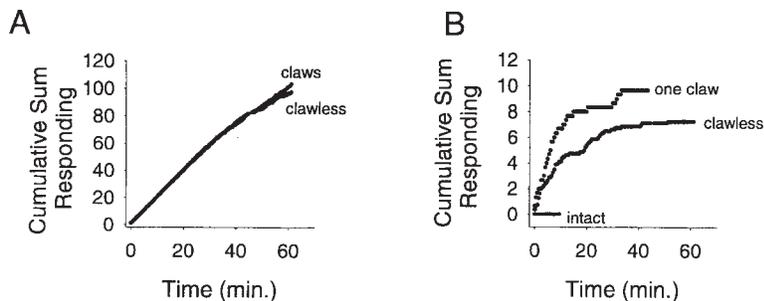


Fig. 6. Groups of small crayfish with both chelipeds present or removed were examined for the tail flip response. There was no difference in the rate of habituation for the total number of tail flips between these two experimental conditions (A). Large crayfish did not exhibit tail flipping upon

initial stimulation, but upon claw removal, they rapidly gained the ability. With one or both chelipeds removed, tail flipping behavior was readily observed for a substantial number of trials until repetitive failures occurred following 50 min of sampling (B).

garding cheliped mass to body mass in behavior of *P. clarkii*, it is logical to hypothesize that the large adults among the cave crayfish would not tail flip upon initial testing. If the crayfish did tail flip then one may also expect that they would demonstrate a rapid habituation. Since blind cave crayfish rely on other senses besides vision and likely have altered neuronal circuits for visual induced escape reflexes, adult blind cave crayfish may differ from *P. clarkii* in tail flipping behavior despite the cheliped-mass to body-mass ratio. Therefore, we examined male adults of the troglotic crayfish, *O. australis packardii*, as isolates and as a group. Only adults were used because there is a higher confidence in species identification for the larger individuals. Other troglotic crayfish co-inhabit the native cave environment and appear similar while in a juvenile stage.

The adult cave crayfish habituated sooner than did *P. clarkii* of the same body size. On average, they took about 15 min to habituate. This was

sooner than the comparably sized *P. clarkii*. In comparison to the adults of *P. clarkii*, these cave crayfish adults were more likely to tail flip, as demonstrated in the cumulative sum responding (Fig. 8). Group studies of behavioral responses of previously isolated *O. australis packardii* were conducted to determine if density has a role in inducing habituation of retreating behaviors upon contact with other individuals. Observations made within the cave environment indicated that, in low-density pools, this species exhibited a high predominance of tail flipping behavior upon contact with another crayfish. But in crowded pools of crayfish (in the cave environment), the tail flipping behavior was less prevalent when two individuals contacted each other. Creating a pool with movable sides allowed for the reduction of the crayfish environmental area with minimal disturbance (Fig. 9A). The total number of interactions (Fig. 9B) and the strength of interaction (Fig. 9C) were recorded for each environment in sequence

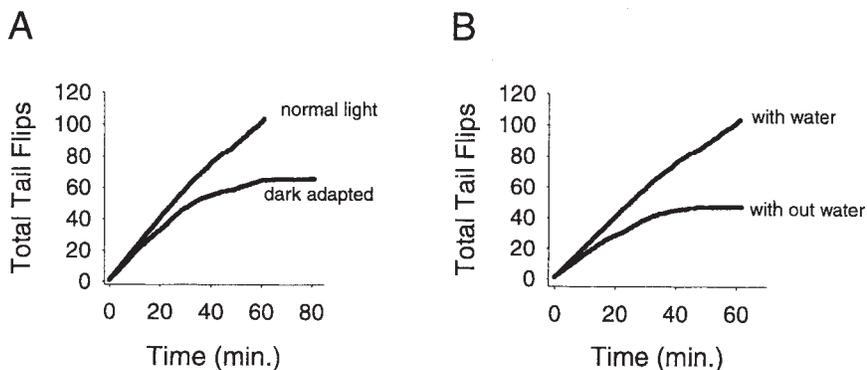


Fig. 7. The effects of dark adaptation on sighted small *P. clarkii* did reveal a shift in the rate of habituation compared to ones maintained in a normal light cycle as shown in the cumulative sum plot (A). Small sighted crayfish tested within

a water-filled tank or within tanks containing only moist substrate revealed that individuals without water did tail flip upon initial testing although they rapidly habituated after 40 min (B).

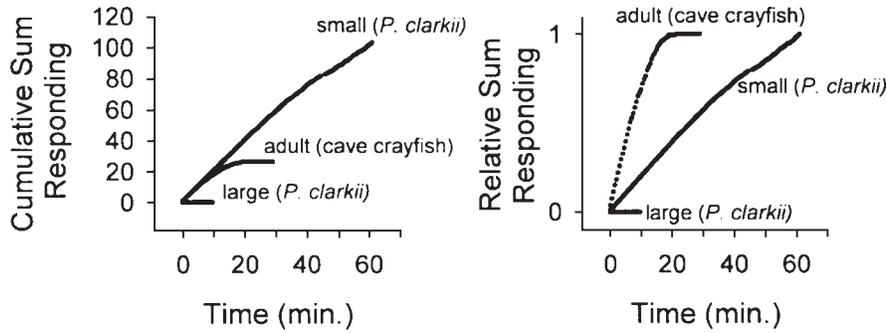


Fig. 8. Adult blind cave crayfish, which are in the same size range as the small *P. clarkii* group, habituated sooner than *P. clarkii* to the tail taps. The group as a whole took about 15 min to habituate. Compared to the large *P. clarkii*,

which never tail flipped, and to the 60 min of tail flipping of the small *P. clarkii* crayfish, the cave crayfish do not behave as adult sighted crayfish nor as ones close in size to *P. clarkii*.

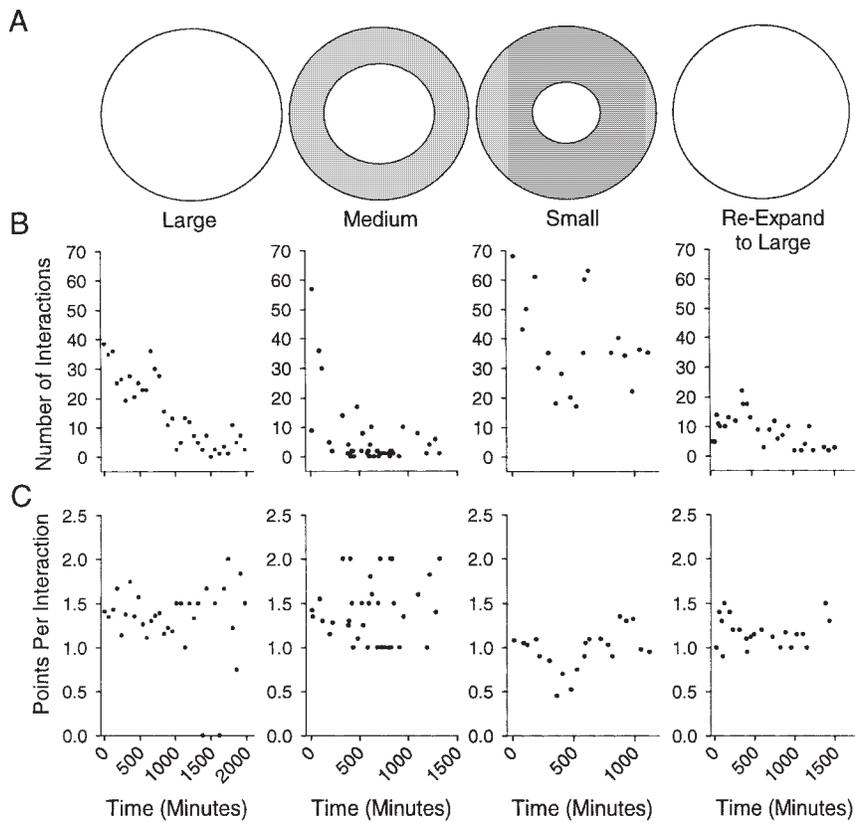


Fig. 9. A combined group of six blind cave crayfish interacted as their environmental pool changed size. An interaction is defined as occurring when two crayfish come in contact with each other. (A) The various sizes of the pool used are illustrated as column indicators for parts B and C: large: 86 cm diameter and 0.581 m²; medium: 45.6 cm diameter and 0.163 m²; smallest: 22.8 cm diameter, 0.041 m². The water depth was kept constant at 8 cm. Dim red light was used for illumination of the experimental room. (B) The number of interactions for the entire group over time decreased in each environment. However, during each tank size reduction, interactions resumed. The smallest environment resulted in in-

teractions for an extended amount of time. The re-expansion of the environment resulted in a decrease in the number of interactions although the interaction strength, as defined in the points of interaction (C), remained relatively constant. The strength of interaction was recorded for each contact. To monitor the type of interaction, an index of interaction was constructed. The scaling of interaction was as follows: 0: No visible reaction when two crayfish touched; 1: Single retreat of a crayfish with no pursuit by the other; 2: Single crayfish retreats with the other pursuing; and 3: Both crayfish visibly retreat by tail flipping.

from large to medium to small areas. Afterwards, a re-expansion of the environment to the large size was given to determine if a reversal in behavior occurred. Seasonal changes make constriction and expansion of pools in the natural cave environment, in which these crayfish were captured, a common occurrence. The majority of all the interactions resulted in one or both individuals tail flipping. Each reduction in the environment area triggered a flurry of movements. This resulted in a larger number of interactions and increased walking or exploration of the area. Next, a settling behavior with reduced movement around the pool occurred. The apparent reduction in exploratory behavior decreased the instances of interaction. To monitor the type of interaction, an index of interaction was constructed. The scaling of interaction was as follows:

0. No visible reaction: crayfish touched, do not move.
1. Single retreat with no pursuit: one crayfish tail flips or runs away, the other does not chase.
2. Single retreat with pursuit: one crayfish tail flips or runs away, the other visibly pursues.
3. Double tail flips: both crayfish visibly retreat.

All interactions of the group and individuals of the cave crayfish took place in dim, red light. This eliminated crayfish seeking shelter as a result of previously observed phototactic behavior (Li and Cooper, 1999, 2001). Since the interaction index remains relatively constant, a significant habituation did not exist among the repetitive interaction strengths within the group for the differently sized environments.

DISCUSSION

This study demonstrates that the probability of the crayfish, *P. clarkii*, to tail flip in response to a touch on the dorsal tail fan is dependent on the size of the animal and the behavioral state of the animal. The larger the animal, the less likely it is to tail flip. Also, a size dependency for an increased tendency for exhibiting habituation to the stimuli with repetitive trials exists. Altering the animal's physical state by autotomizing the chelipeds increases the tail flip response in larger animals. Altering the environment, such as to one with little water depth, also can cause crayfish to respond differently to the stimulus. For example, small crayfish will habituate more rapidly when placed in shallow water. Observations of adult crayfish

of a species adapted to live in cave darkness revealed that they are more likely to tail flip than sighted adults of a different species. When the troglotic crayfish were examined within a group, they exhibited a decrease in general movement over time. Thus, interaction instances decreased. However, the probability to tail flip remained constant in spite of the decrease in interactions.

Earlier studies examined the plausible mechanisms behind habituation of the tail flip response based on size (Fricke, '86). He tested animals less than 2 cm and greater than 10 cm in length. The present study fills in the size ranges of animals tested. Also, the study expands on the latest hypotheses concerning the habituation of the crayfish tail flip response.

The latest hypotheses center around the increased prevalence of depression-prone synapses as the animals mature to full adults (Yeh et al., '97). It does appear that the synaptic circuitry can be influenced by long-term modulation based on an animal's behavioral state (Yeh et al., '97). The results of Yeh et al. ('97) show that the neuro-modulator, serotonin, enhanced the response to sensory stimuli to the lateral giant neurons within a semi-intact preparation. It is suggested that different responses among social levels following exogenous application of serotonin is due to variation in the various serotonin receptor subtypes present. This type of plasticity requires time to alter receptor subtype receptivity during encounters. Considering the real world ecological arena, an animal's best interest would not be met by having a set response based on its current social status because at any moment, a larger crayfish may intrude the social structure and challenge a smaller crayfish that is dominant in its own group. In such a situation, immediate retreat and/or submissive posture assumption would help a smaller crayfish to survive and be selected for this trait.

Monitoring the occurrences of tail flips before and after the animals autotomized their chelipeds tests the idea of rapid alterations in the tail flip response. There is a substantial increase in tail flipping behavior of the large adults and a slight increase in the medium sized crayfish that do not retain their chelipeds, as compared to a control group with both chelipeds intact. The observation by Lang et al. ('77) that dominant lobsters induced to autotomize their chelipeds will switch to subordinate behavior supports this finding. These results indicate that there is a rapid alteration in the telson induced response to tail flip command circuitry that enhances the ability of the animal

to tail flip. Thus, it is unlikely that this rapidity in the alteration in responsiveness is due to receptor up or down regulation or alteration in synaptic structure.

The current findings on tail flip habituation show responses are altered rapidly by removal of the chelipeds. This suggests a hormonal effect and/or direct neural synaptic regulation of the sensory-motor reflex (Listerman et al., 2000; Strawn et al., 2000). Possibly, neuromodulators are responsible for such behavioral alterations. A likely candidate is octopamine. The neural processes of neurohumoral cells that project into the chelipeds contain octopamine and may release it into the hemolymph upon cheliped removal (Heinrich et al., '99). As for the 5-HT containing neurons, these octopaminergic neurons may function as "gain-setters," altering the output of neuronal circuits (Schneider et al., '96; Ma et al., '92). Kravitz's group (Livingston et al., '80) found injections of octopamine into a lobster result in the animal mimicking the submissive postures observed during social interaction. This is consistent with the present suggestion that octopamine is released upon claw removal to cause a rapid switch in the tail flip response.

Removing the water from the testing tank provides information on environmental influences to the tail flip response. Water removal may have increased the effective weight of the animal or triggered other sensory receptors. Crayfish were given 10 min to accommodate to the new environment of reduced water before testing was initiated. During the 10 min, the animals became active. This behavior implies that they are responding to the environmental alteration. Furthermore, crayfish that stay out of water for too long will perish from desiccation. Thus, one is led to believe crayfish are selected to react to the absence of water. In spite of this, small crayfish tail flip for a significant number of times (30) before habituating. This behavior continued despite sometimes resulting in an animal landing on its back exposing the soft abdominal underside that is vulnerable to attack. Yet, the animals out of water did habituate more quickly to the stimulus. This study suggests that the environment can alter the animal's tail flip behavior.

The long-term effects of a unique environment on tail flip behavior were studied with the unsighted cave crayfish *O. a. packardii*. Cave crayfish provide a multifold purpose. It would not be surprising if tactile and/or chemosensory systems were enhanced in the unsighted cave crayfish. In

fact, there is suggestive evidence that this species has enhanced numbers of olfactory projection neurons (OPN). OPN are second-order neurons that have their cell bodies within cluster #10 of the central brain (Cooper et al., '98, 2001; Schmidt, '97.). In order to avoid predation, adults may be resistant to habituation, due to their adaptations to a cave environment. Of course, factors such as being in a high-density situation may alter behavioral states for greater energy efficiency. Thus, these studies begin to answer these questions under laboratory conditions.

Also, the *O. a. packardii* allow determination of whether ratios of cheliped length to body length are a correlative factor across species. The largest adults found within the cave species display a cheliped length to body length ratio that is greater than in adult *P. clarkii*. This ratio would suggest that cave crayfish are less likely to tail flip as adults. However, testing reveals that the large cave crayfish demonstrate the ability to tail flip. This ability occurs independent of body proportions.

The data on body proportions would benefit from a larger number of samples of data on mass of the chelipeds to body mass. The study by Fricke ('86) on the sighted crayfish, *Orconectes virilis*, can be used for comparison. However, a sample of that size for the cave crayfish would have meant damaging a larger number of this potentially environmentally threatened species of cave crayfish. The cave crayfish would have a reduced defensive ability (i.e., survival) upon returning them to their native environment. Thus, only one chela was removed from each size range of animals to obtain the mass of chelipeds.

The results obtained with the adult cave crayfish clearly indicate significant differences in habituation rates from those published by Fricke ('86) for *Orconectes virilis* and data presented in this study for *P. clarkii*. In addition, the group data of interaction occurrences and interaction strength indicates that the cave crayfish maintain tail flipping following repetitive interactions within a particular environment. When the environmental area was reduced, the interaction rate increased and later decreased. An exception occurred when the space was extremely reduced. In this case, an animal continued to show tail flipping behavior upon contact with another individual. Following a disturbance, the animals moved around the newly delimited territory. Over time, they dug an indentation within the sand as a settling spot. Such settling behavior reduced the number of interactions within the group study.

When an interaction did occur, one of the individuals would normally tail flip to avoid the interaction. Further detailed studies on social behaviors in the cave crayfish are just beginning to determine if the behavioral variations are comparable to those of sighted crayfish (Li et al., 2000; Li and Cooper, 2001; Cooper et al., 2001).

In summary, the results indicate that the behavioral state of the crayfish can result in rapid and long term alterations in the tail flip response and in habituation rates to repetitive stimuli. This ability to show plasticity in gain setting occurs in adults of sighted crayfish. This alteration may be due to hormonal regulation, but more work is required before fully addressing this possibility. The intact adult cave crayfish (*O. a. packardii*) are more likely to tail flip than are intact adult *P. clarkii*. This study demonstrates that factors other than age and maturity may influence tail flip behavior upon stimulation of the dorsal telson in adults of some species.

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