

SHORT COMMUNICATION
**INHIBITION OF ESCAPE TAILFLIP IN CRAYFISH DURING
BACKWARD WALKING AND THE DEFENSE POSTURE**

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Accepted 5 April 1990

A major question in neuroethology is how animals coordinate transitions between incompatible behavior patterns. The recent demonstration that the lateral giant (LG) tailflip is inhibited during eating (Krasne and Lee, 1988) suggested to us that inhibition may play a general role in preventing maladaptive transitions between incompatible behavior patterns. We wished to determine whether the LG tailflip is inhibited during other incompatible behavior such as backward walking and the defense posture.

Different behavior patterns are incompatible because they move the same body parts differently and because they are adaptive responses to different sensory stimuli. This is the case with backward walking, defense posture and escape tailflip in crayfish. Backward walking is frequently observed among crayfish living communally in a laboratory aquarium, often in response to stimuli that signal potential rather than immediate danger, or in response to possible conflict with conspecifics. These stimuli include tactile stimulation of the antennae, approach of threatening visual stimuli, illumination of the eyes and/or illumination of the caudal photoreceptors in the last abdominal ganglion (Welsh, 1934; Kovac, 1974; Edwards, 1984; Simon and Edwards, 1990). The defense posture is a threat display that occurs in response to looming visual stimuli and consists of raising and spreading the chelipeds towards the stimulus, raising the cephalothorax by extending the legs in a widened stance, and extending and flattening the abdomen against the substratum. Escape tailflip occurs in response to immediately threatening stimuli, to direct attacks or blows, or during conflict with conspecifics (Wine and Krasne, 1972, 1982). The upwardly directed, somersault tailflip can be triggered by a sharp tap on the dorsal abdominal surface; the tap excites mechanosensory afferents and mechanosensory interneurons that converge on the bilaterally paired LG interneurons. These cells act as 'decision neurons' for the behavior (Zucker, 1972; Olson and Krasne, 1981).

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Key words: crayfish, backward walking, defense posture, tailflip, behavioral inhibition.

To look for inhibition of LG tailflips during walking and defense, we measured the frequency of occurrence of tailflips over a range of stimulus intensities under control conditions and when the animal was walking backwards or displaying the defense posture.

Adult crayfish (*Procambarus clarkii*, 7–10 cm from rostrum to telson) were obtained from a commercial supplier (Waubun Laboratories, Schriever, LA) and maintained communally in freshwater aquaria. Complete sets of experiments for walking and defense posture were performed on each member of two sets of five animals.

LG-mediated tailflips were evoked by electrical stimulation (1.5 ms voltage pulse of variable amplitude) of a sensory nerve of the last abdominal ganglion *via* a set of implanted electrodes. Twenty-four hours before an experiment, a crayfish was chilled in an ice bath until it became quiescent, when a pair of silver hook electrodes was inserted through a slit in the translucent soft cuticle and placed around a fourth nerve of the sixth abdominal ganglion. The electrode leads were connected to the pins of a microchip socket fixed to the dorsal thoracic carapace. Before an experiment, light-weight, flexible leads from a stimulus-isolated pulse generator were connected to the microchip socket; the leads allowed the animal to behave in a fully unrestricted manner.

LG-evoked tailflips are identifiable by their upward direction and short response latency (<10 ms from time of stimulus presentation; Krasne and Wine, 1984). We obtained the response latency of one animal from both fast flexor muscle electromyograms (EMGs) and bath-recorded field potentials. EMGs were recorded *via* a set of electrodes inserted through the soft abdominal ventral cuticle into the fast flexor muscle. Field potentials were recorded from a pair of wire electrodes placed in the bath at either end of the experimental aquarium. Since the two methods gave identical results (Fig. 1), we used the latency of the bath-recorded field potentials for the remaining animals. Of the approximately 800 evoked tailflips all were upwardly directed and had latencies of less than 10 ms. We conclude that we consistently evoked LG-mediated tailflips.

Illumination of the ventral surface of the crayfish with bright white light excites the eyes and the caudal photoreceptor and provides a reliable stimulus for backward walking (Welsh, 1934; Edwards, 1984; Simon and Edwards, 1990). The light source was a 200 W tungsten lamp placed beneath a clear aquarium filled to a depth of 7 cm with fresh distilled water. The animal was free to move anywhere in the aquarium. The light was placed beneath the quiescent animal at the beginning of each trial. All backward walking experiments were carried out under dim red light to prevent visual stimuli from affecting the animal's behavior.

Crayfish remained undisturbed in the darkened aquarium for at least 15 min before trials began. Stimulus pulses were delivered while the animal rested in the dark or after it had received 10 s of ventral illumination. Animals were considered to be walking backwards if they had begun to move at least 2 s before the shock. Trials with no light and with light stimuli were given randomly, and stimulus pulse amplitudes were changed irregularly over a range between zero and a maximum

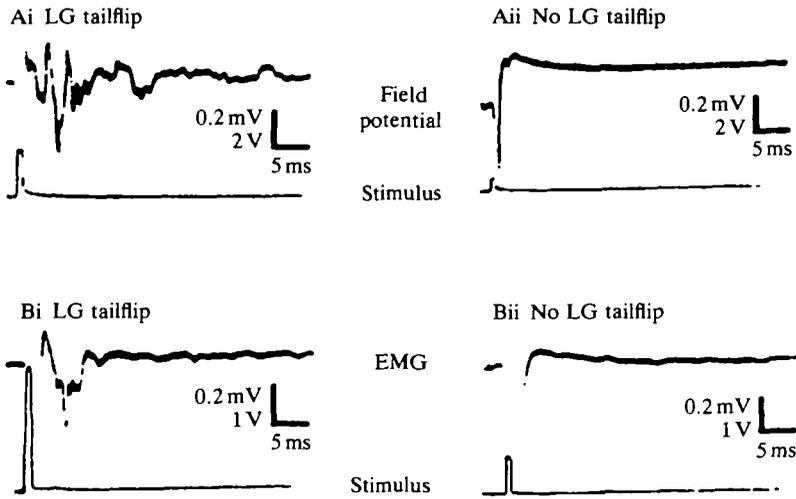


Fig. 1. Bath-recorded field potentials and fast flexor electromyograms (EMGs) during a tailflip. (A) Field potentials (top) following a stimulus pulse (bottom) to the sensory nerve of A6 that succeeded (Ai) and failed (Aii) to evoke a tailflip. (B) EMG potentials (top) evoked by a similar stimulus (bottom) that succeeded (Bi) and failed (Bii) to evoke a tailflip. LG, lateral giant.

level sufficient to obtain tailflips consistently under all conditions. Inter-trial intervals were at least 5 min to prevent habituation of the tailflip response (Krasne, 1969; Reichert and Wine, 1983). Each animal experienced at least four trials at each stimulus level and condition; the average number was 7 ± 2.1 (s.d.).

The response frequency (the ratio of the number of tailflips to the number of trials) was recorded for each stimulus voltage under each of three stimulus conditions: the animal rested in the dark (Dark), the animal was illuminated but remained stationary (Light/No BW), and the animal was illuminated and walked backwards (Light/BW). The stimulus voltages recorded for each animal were normalized to the value at which a response frequency close to 50% (mean = 57%) occurred in the dark condition; this stimulus level was defined as one stimulus unit and all other stimulus levels were scaled proportionately.

The tailflip response frequencies varied with the stimulus level in a sigmoidal fashion under all three conditions, such that at high levels a tailflip occurred on 100% of trials (Fig. 2A). The curves for the Dark condition and the Light/No BW condition were nearly identical, whereas that for the Light/BW condition was displaced to a higher range of stimulus intensities. At a stimulus level of 1.3 units, crayfish under the Dark and the Light/No BW conditions tailflipped on over 90% of trials, whereas they responded on only 50% of trials when they were walking backwards. This result indicates that the tailflip response to root shock was inhibited when the crayfish was walking backwards in response to ventral illumination, but not when ventral illumination failed to evoke walking.

The defense posture can be reliably elicited by the frontal approach (looming)

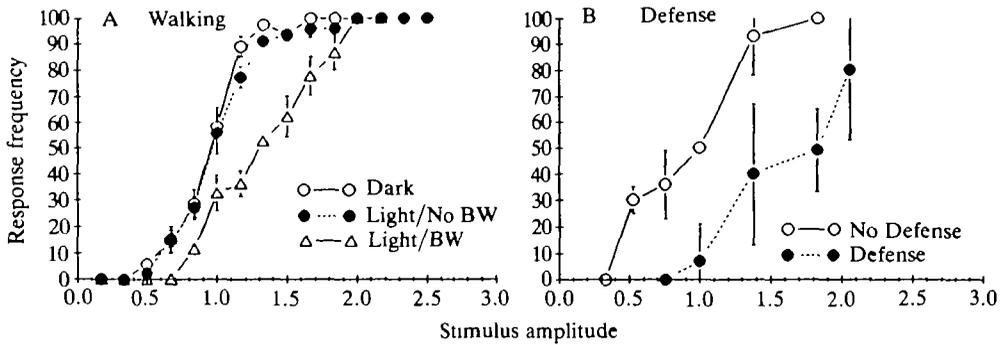


Fig. 2. Tailflip response frequencies as a function of stimulus amplitude during backward walking and display of the defense posture. (A) Averages (\pm s.d.) of response frequencies of five animals during light-evoked backward walking (Light/BW), while quiescent in the dark (Dark) and while quiescent in the light (Light/No BW). (B) Average (\pm s.d.) response frequencies of five animals during the defense display (Defense) and while quiescent in the dark (No Defense).

of any target larger than the animal; we used one end of a 30 cm \times 5 cm \times 3 mm rectangular piece of wood that was manipulated by hand from behind a screen. The area surrounding the aquarium was darkened while the part of the aquarium in front of the animal was illuminated from behind the animal with a spot of light. The target was lowered into the light 30 cm from the animal and advanced broadside towards the crayfish at approximately 5 cm s⁻¹; this nearly always evoked display of the defense posture. The shock to the abdominal nerve was delivered when the crayfish was in full display (Defense), with abdomen extended, telson and uropods flattened, cephalothorax elevated and chelipeds extended and raised well above the cephalothorax. These stimuli were interleaved randomly with control stimuli (No Defense) in which the animal was shocked while quiescent in the darkened aquarium. As above, response frequencies (based on at least four responses) were recorded for each stimulus voltage and condition (Defense/No Defense). The stimulus amplitudes for each animal were normalized as described above.

Tailflip response frequency increased with stimulus amplitude over a higher range of stimulus intensities during display of the defense posture than when the animal was quiescent (Fig. 2B). As during walking, the tailflip response frequency to stimuli of 1.4 units in amplitude decreased from the control level of 90% to 40% during the defense display. The tailflip system is inhibited during defense as well as during walking.

The inhibition of the LG-evoked tailflip that occurs during backward walking could result from the walking motor program, from reafference during walking, or from an independent neural source that is also excited by ventral illumination. In the last case, the threshold for that independent source would have to co-vary with that for walking, since inhibition occurred consistently only when the light stimulus evoked backward walking. Such independent co-variation of the walking

and tailflip inhibitory thresholds seems unlikely, which argues against independent neural pathways for the two effects. Electrophysiological evidence supports the first two possibilities. The LG escape system is inhibited by a population of proprioceptive interneurons that are excited by walking leg movements (Fricke *et al.* 1982; Fricke and Kennedy, 1983). Preliminary evidence indicates that the LG escape system is centrally inhibited during stimulation of interneurons that initiate the backward walking motor program (Edwards *et al.* 1988). These results indicate that the LG escape system is inhibited both centrally and through reafference during walking. By contrast, nothing is currently known about the source of LG tailflip inhibition during the defense display, although inhibitory proprioceptive interneurons may also be excited by leg movements that occur as the cephalothorax is elevated.

The LG tailflips are also suppressed during feeding, during restraint and by inhibition of the LG evoked by an impulse in the medial giant (MG) interneuron and in the LG itself (Krasne and Lee, 1988; Krasne and Wine, 1975; Roberts, 1968). For some behaviors it is clear that LG inhibition acts to prevent reafferent excitation of the LG, such as during tailflip or backward walking (Kennedy *et al.* 1980; Fricke *et al.* 1982; Fricke and Kennedy, 1983). Performance of other behaviors, such as feeding and defense, should generate no reafferent stimuli for the LG tailflip, and so inhibition would raise the response threshold of the LG to exafferent stimuli during those activities. This would appear adaptive for both behaviors, since an LG tailflip during either one is likely to have undesirable consequences. An LG tailflip pitches the crayfish up and forwards (somersault), which would be towards any threat that would elicit the defense display (Wine and Krasne, 1972). Feeding frequently occurs in the presence of conspecifics, and an untimely tailflip might pitch the animal away from the food source.

This work was supported by NIH Research Grant NS21136.

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