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Neuroendocrine and behavioral responses to weak electric fields in adult sea lampreys (*Petromyzon marinus*)

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Abstract

We characterized the behavioral and neuroendocrine responses of adult sea lampreys (*Petromyzon marinus*) to weak electric fields. Adult sea lampreys, captured during upstream spawning migration, exhibited limited active behaviors during exposure to weak electric fields and spent the most time attached to the wall of the testing arena near the cathode (−). For adult male sea lampreys, exposure to weak electric fields resulted in increased lamprey (l) GnRH-I mRNA expression but decreased lGnRH-I immunoreactivities in the forebrain, and decreased Jun (a neuronal activation marker) mRNA levels in the brain stem. Similar effects were not observed in the brains of female sea lampreys after weak electric field stimulation. The influence of electroreception on forebrain lGnRH suggests that electroreception may modulate the reproductive systems in adult male sea lampreys. The changes in Jun expression may be associated with swimming inhibition during weak electric field stimulation. The results for adult sea lampreys are the opposite of those obtained using parasitic-stage sea lampreys, which displayed increased activity during and after cathodal stimulation. Our results demonstrate that adult sea lampreys are sensitive to weak electric fields, which may play a role in reproduction. They also suggest that electrical stimuli mediate different behaviors in feeding-stage and spawning-stage sea lampreys.

Keywords: Sea lamprey; *Petromyzon marinus*; Electroreception; GnRH

Introduction

Electroreception has evolved in a number of vertebrate species occupying diverse aquatic habitats (Bullock et al., 1983; Caputi and Budelli, 2006; Collin and Whitehead, 2004). Most studies on electroreception have been focused on electrolocation and electrocommunication in weakly electric fish due to the distinct and tractable behaviors they display such as the jamming avoidance responses (Zupanc and Bullock, 2006), and on how environmental and neuroendocrine changes alter the electrophysiology of the electroreceptors, the electric organ discharge (EOD), and the nervous system involved in electroreception (Stoddard et al., 2006). Passive electroreception is less emphasized and attention has traditionally been focused on cartilaginous fishes (Collin and Whitehead, 2004). These species use electroreceptors to detect weak bioelectric fields produced by other animals' skin and muscular contractions (Caputi and Budelli, 2006), suggesting that they may use electroreception for prey detection and predator avoidance. Studies also suggest that electroreception may be used during migration and geomagnetic orientation, and complex social behaviors in elasmobranchs (Collin and Whitehead, 2004; Demski, 1989; Kalmijn, 1978, 1982; Sisneros and Tricas, 2000; Tricas et al., 1995).

Sea lampreys are one of the most ancient lineages of vertebrates which possess electroreception (Bullock et al., 1983; Collin and Whitehead, 2004). Electrophysiological studies have documented that adult sea lampreys are responsive to weak, low frequency electrical fields (Bodznick and Northcutt, 1981; Bodznick and Preston, 1983; Ronan and Bodznick, 1986). Early research reported spike potentials in the water surrounding the head region of sea lampreys (Kleerekoper and Sibakin, 1956a,b, 1957). These authors suggested that these signals, in

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combination with as yet unidentified electroreceptors, could function as an active electroreceptive system in prey detection, rather than as a passive system as subsequently shown. Bodznick and Northcutt (1981) demonstrated the neural substrates for electroreception in sea lampreys, and this led to subsequent identification of epidermal electroreceptors, previously described as end buds, in adult sea lampreys and silver lampreys (*Ichthyomyzon unicuspis*) (Bodznick and Preston, 1983; Ronan and Bodznick, 1986). The behavioral and neuroendocrine responses of parasitic-stage sea lampreys to weak electric fields were also documented (Chung-Davidson et al., 2004).

No clear functions have been identified for electroreception in adult sea lampreys. During the short adult life stage, sea lampreys cease feeding in the early spring and migrate into rivers to spawn and die (Applegate, 1950; Hardisty and Potter, 1971). Olfactory cues (pheromones) play critical roles during spawning to attract mates and perhaps release spawning behavior in sea lampreys (Johnson et al., 2006; Li et al., 2002; Siefkes et al., 2005). However, recent observations indicate that pheromones alone are not sufficient to retain sea lampreys in the nest site when they arrive at the spawning ground (Johnson et al., 2006). It is reasonable to hypothesize that electrical cues arising from adult sea lampreys may be used to detect conspecifics over short distances. Since behavioral responses to weak electric fields have not been characterized in adult sea lampreys, we sought to examine the effects of weak electric field stimulation on behavior and the neuroendocrine system in adult sea lampreys.

Materials and methods

Collection and maintenance of animals

Adult sea lampreys (body mass 200–350 g with an average body length of 48 cm) were captured during upstream spawning migration by agents of the USFWS from tributaries of Lakes Huron and Michigan. They were shipped to East Lansing, Michigan, and kept in a 1325-liter fiberglass tank (L × W × H: 122 × 122 × 89 cm) with flowing water at 16.6–16.8 °C [aerated and filtered deep-well (300 ft) water, conductivity 510.715 ± 15.022 μS/cm (mean ± S.E.M)]. Animals were used within two weeks and were not fed. Animal handling procedures were approved by the University Laboratory Animal Resources at Michigan State University.

Experiment 1. Behavioral responses to weak electric fields in adult sea lampreys

Behavioral test

Ten adult sea lampreys were subject to the behavioral test as described in Chung-Davidson et al., 2004. A circular plastic divider (Radius: 1 m; Height: 1 m) within a 2 × 2 × 1.5 m^3 plastic tank in a wooden housing 1 m above the ground was used for behavioral tests (see Suppl. Fig.). One metal electrode (BNC-to-2 mm pin, World Precision Instruments, FL, USA), embedded in 1% agar in a 5 ml plastic syringe, was mounted on one side of the wall of the circular tank, and a second electrode on the opposite wall. The electric field at 17 locations within the circular tank was measured (see the location of number 0–16 in Suppl. Fig.) by two electrodes held 1 cm apart and connected to a Tektronix 2213A 60 MHz oscilloscope (Tektronix Inc., TX, USA). The electric field generated was confirmed to be homogeneous across the whole circular arena except around the electrode not delivering the pulses. The cathode (−) and anode (+) were randomly assigned to the electrodes for each test. Prior to the behavioral test, each lamprey was introduced into the testing arena and allowed to acclimate for 5 min. Two video cameras mounted on top of the tank at a 90° angle from each other were used to record the behavior. The behavioral test started and ended simultaneously with the rectified voltage outputs generated by the ‘Scope’ computer program (AD Instruments, CO, USA) and the PowerLab AD/DA converter (AD Instruments; 500-millisecond delay, 150-millisecond duration, 500-millisecond interval, 10 rectangular wave pulses/10 s/sweep, 10 sweeps total for each amplitude tested, modified from Bodznick and Northcutt, 1981; Bodznick and Preston, 1983; Kleerekoper and Sibakin, 1956a,b, 1957). For each fish, a baseline behavioral test was performed with the stimulation amplitude set at 0 mV first. The orders of whether the fish received anodal (+) or cathodal (−) stimulation first and the amplitude were randomly assigned; amplitude tested: 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8, 2, 3, 4, 5, 10, 20, 200, and 500 mV, and 1, 2, 5, and 10 V. For example, if anodal stimulation was performed first, the sequence of anodal field amplitudes (0.1 mV to 10 V) was randomly drawn and the behavioral test performed. After the tests for anodal stimulation, another baseline behavioral test was performed, and the sequence of cathodal field amplitudes (−0.1 mV to −10 V) was randomly drawn and the test performed. If the lamprey continued to swim after the stimulation stopped, data were collected until the fish attached to the wall.

Behavioral parameters and statistics

Several parameters were used to define the behavioral responses: *latency to move,* the time from the start of the test to the first noticeable movement of the lamprey other than breathing, either swimming or twitching while remaining attached to the tank wall; *latency to swim,* the time from the start of the test to the first swimming behavior; *duration of swimming,* the total time spent in swimming during a behavioral test; *swimming distance*, the total travel distance from the start to the end of the test; *duration of twitching,* the time spent attached to the tank wall with body movement in each test; *latency to stop,* the time from the end of the electric stimulation to the first attachment; *duration of attachment*, total time spent attached to the wall without body movement in each test. To test whether the lamprey preferred a certain location after weak electric field stimulation, we marked the *location preference* of the test fish immediately before and after each test. We defined that a preference for specific electrode was observed when the fish was located within a 50 cm radius of this particular electrode, since empirical measurements demonstrated that the ”active space” (the area or volume within which the signal can evoke responses from an organism) for electric modality was limited to a radius of 50 cm to several meters only (Hopkins, 1988; Moller and Bauer, 1973; Squire and Moller, 1982). Fish located outside the 50 cm radius of any electrode were recorded as exhibiting no preference for any electrode.

Statistical analyses were performed by the computer software StatView (SAS Institute, NC, USA). For most behavioral parameters, a one-way ANOVA test for repeated measurement (anodal vs. cathodal stimulation) was used to compare the difference among stimulation field intensity, including a no-stimulus baseline, and different voltage amplitudes. Bonferroni/Dunn’s post hoc tests were performed if the ANOVA results showed significant differences among groups ($p < 0.05$). For all or none events, non-parametric Kruskal–Wallis tests were used for statistical analyses followed by Mann–Whitney *U* tests

Table 1
Location preference of adult sea lampreys after weak electric field stimulation

		Location preference		
		Anode (+)	Cathode (−)	No preference
Stimulation	Anodal (+)	42	81	44
	Cathodal (−)	59	99	18
	None	11	13	1

Weak electric field stimulation induced a location preference in adult sea lampreys ($\chi^2 = 20.281$, dF = 4, $p < 0.0005$). In a total of 368 tests, fish were located within 50 cm radius of the cathode (−) in 193 cases (81 + 99 + 13), and in 112 cases (42 + 59 + 11) fish were located within 50 cm radius of the anode (+) when no or any (+ or −) electric field stimulation was applied. Only in 63 tests (44 + 18 + 1) were fish located outside the 50 cm radius of both electrodes at the moment when the stimulation stopped or when no electric field stimulation was applied.