GREAT LAKES FISHERY COMMISSION

1996 Project Completion Report[1]

Olfactory Mucosa Neural Response in Prolarval Sea Lampreys: A Basis for Pheromone Communication During Settlement to Larval Habitat.

by:

Barbara Zielinski[2]

[2]Associate Professor, Dept. of Biological Sciences
University of Windsor
Windsor, Ont. N9B 3P4

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Report to the Great Lakes Fishery Commission:
Olfactory mucosa neural responses in prolarval sea lampreys: a basis for pheromone communication during settlement to larval habitat.

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Submitted by: Barbara Zielinski, Associate Professor, Dept. of Biological Sciences, University of Windsor, Windsor, Ont. N9B 3P4.

Submitted to: Randy Eshenroder, Senior Scientist, Great Lakes Fishery Commission, 2100 Commonwealth Blvd., Suite 209, Ann Arbor MI 48105-1563.

Objective: To substantiate findings from 1995 that showed olfactory mucosal neural response from prolarval sea lampreys to stimulation with solutions containing specific molecules and to water conditioned by conspecifics.

Background: The olfactory mucosal neural response displays action potentials that are fired from olfactory receptor neurons following the application of a test solution. It is a direct indication of the effectiveness of a compound to stimulate olfactory neural activity. Our work in 1995 showed responses to the amino acid, L-arginine; and the bile acid, taurocholic acid. Low amplitude responses were observed following the application of water conditioned by ammocoetes and to water conditioned by familiar siblings. Concentration dependent high amplitude responses were observed following the application of water conditioned by unfamiliar nonsiblings. The fact that the response magnitude to unfamiliar nonsiblings was greater than responses to familiar siblings, lead me to propose that prolarvae are able to recognize same age conspecifics on the basis of relatedness or familiarity. According to this hypothesis, if stage 17 prolarvae responded to unfamiliar nonsiblings rather than familiar siblings during nest abandonment, then prolarvae would select settlement sites with unrelated conspecifics. If the gene pool dispersed to sites with unrelated conspecifics, then the gene pool survival may be greater than if related prolarvae recruited to the same settlement site.

Materials and Methods:
Prolarvae: In the spring of 1996, lampreys were caught in traps in the Ocqueoc River and were maintained in the lab at the Lake Huron Biological Station at 18°C. These were spawned with appropriate crosses and eggs were cultured at 18°C. Eggs were cultured so that olfactory mucosal neural responses included tests of relatedness and familiarity. The following groups were cultured: sibling familiar*, sibling unfamiliar*, unrelated familiar, unrelated unfamiliar*, half sibling familiar, half sibling unfamiliar*. The groups with an asterisk(*) developed to stage 17 prolarvae. Familiarity between two groups was achieved by applying water from one group to the other group. A very low number of prolarvae survived in two groups, unrelated familiar and half sibling familiar. Responses

from these are shown in Table 1, but are not included in Figure 2. Later attempts to culture eggs from St. Mary's spawners and from Atlantic lampreys failed.

Olfactory mucosal responses from 17 prolarvae were recorded at the Lake Huron Biological Station. The experimental setup was the same used in the 1995 Final Report to the Great Lakes Fishery Commission. The preparations were stable for recordings for over an hour. The following solutions were tested: petromyzonol sulfate (donated by Dr. P. Sorensen), chemicals from Sigma (St. Louis): taurocholic acid, L-arginine, the L-arginine analogues: Nω-nitro-L-arginine and N^G-methyl-L-arginine. Water conditioned by conspecifics was applied directly. A drop of the test solutions was applied to the dish with the prolarva. The dilution factor for this drop in the sample dish was approximately 1000X

Calculations: The spike amplitude was measured and the response magnitude was calculated by comparing the amplitude to the average response value to 10^{-6} M L-arginine.

Results and Discussion: Examples of the olfactory mucosal neural responses are shown in Figure 1 and the averaged values for the response magnitudes of olfactory mucosal neural responses are shown in Figure 2.
Petromyzonol sulfate: The responses were strongest to petromyzonol sulfate (10^{-12} M). Slightly lower values to a higher concentration (10^{-10} M) of petromyzonol sulfate, were similar to responses to the bile acid taurocholic acid (10^{-9} M). There was a degree of cross-adaptation between these to bile compounds, as the application of taurocholic acid prior to petromyzonol sulfate diminished the amplitude of petromyzonol sulfate response. These results show that the prolarvae respond to pheromones. The olfactory receptor neurons respond with summated action potentials to extremely low concentrations of petromyzonol sulfate; 10^{-14} M, when the dilution of the preparation is considered. Considering the sensitivity of the prolarval olfactory receptor neurons to petromyzonol sulfate, this pheromone may be an attractant for prolarvae during settlement. Stage 17 prolarvae contain bile in the liver and presumably excrete traces of petromyzonol sulfate.
Water conditioned by conspecifics: Prolarvae The response magnitudes to water conditioned by same age conspecifics support the view that prolarvae respond to pheromones. The question of differing responses depending on relatedness and familiarity is less clear, as response magnitudes were similar regardless of relatedness and familiarity. There was considerable variation among individual responses (Table 1), that was not evident in the averages displayed in Figure 2. These results differ from those in 1995 that suggested higher responses to unfamiliar unrelated prolarvae compared to familiar sibs. However, Figure 2 does show slightly stronger responses to unfamiliar siblings compared to familiar siblings. The averaged response magnitude to related prolarvae was greater than to unrelated prolarvae. This is the first study to compare olfactory physiological responses based on relatedness and familiarity, and supports previous behavioral tests with salmonids (eg. T.P. Quinn & C.A. Busack, Anim. Behav. 1985 33;51-56), amphibians (eg. B.W. Waldman J Comp Physiol. A 1985 156:565-577) and rats (reviewed by P.G. Hepper, Biol. Rev. 1986 61:63-93) that showed preference favoring relatedness. The small scale of these experiments may have been a factor in clearly demonstrating trends.

Eggs and ammocoetes: The low response magnitude to water conditioned by ammocoetes is consistent with results from 1995. This is surprising, as this water would have contained the effective chemostimulant, petromyzonol sulfate, a product in the bile of ammocoetes. However, when one considers that prolarvae should recruit to a habitat with conditions that are appropriate for their small size, to settlement sites with conspecifics that have a similar size, it is understandable that the response magnitude to prolarvae is greater than to ammocoetes. Consistently strong responses were observed following the application of water conditioned by stage 12 eggs. These eggs have not reached liver formation and would not be producing endogenous bile acids, however, the egg coverings contain molecules from parental origin with chemostimulatory properties. These experiments suggest that pheromonal communication is not limited to petromyzonol sulfate, that there may be other compound(s), present in eggs and potentially in prolarvae that are also pheromones.
Nitric oxide: The inhibition of L-arginine responses by analogues that are reversible blockers of nitric oxide synthase, Nω-nitro-L-arginine and N^G-methyl-L-arginine, supports the involvement of the intercellular messenger nitric oxide in olfactory transduction of L-arginine. Nitric oxide is an ubiquitous messenger that is active in numerous physiological processes in vertebrates and invertebrates. The application of these blockers may transiently disrupt prolarval olfactory responses to L-arginine and would undoubtedly affect essential physiological activity of nontarget organisms.

Conclusion: These findings support the development of pheromonal attractants or repellents to disrupt the process of settlement following nest abandonment. The sensitivity that prolarval olfactory receptor neurons display toward petromyzonol sulfate, point to the testing that pheromone. Results from this study also suggest that sea lamprey prolarvae respond to other unidentified pheromones.

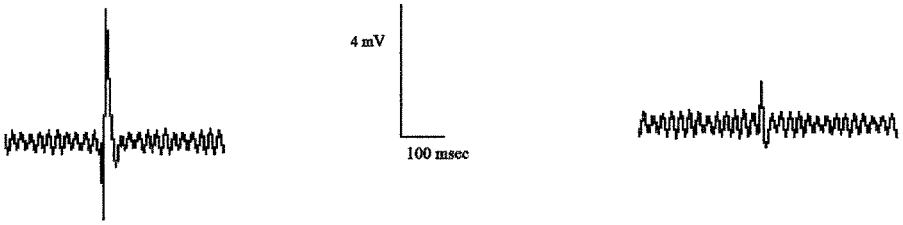
Table 1: Magnitude of response (units from physiograph) to water conditioned by conspecifcs

L-arginine	Sibling Familiar	Sibling Unfamiliar	Unrelated Unfamiliar	Unrelated Familiar	Half sibling Unfamiliar	Half sibling Familiar
9	4	7	3	1.5	7	7
6	12	5.5	9		8	3
6.25	5	11	3		5	12
1.25	3	4.5	12		3	7
2	4	6	11		12	8
2.5	11	1	7.5		9	
6	12	3	9		2.5	
5.5	2.5	5	12		2	
	4		3.5		3	
	1.5		8		10	
	4		1		5	
	3		9		3	
	3		1		2	
	8		3		0	
	3				5.5	
	0		0			
	2		0			
	8		2			
	7		3.5			
	0		3			
	3		7			
	10		9			
	5		1			
	2		3			
	12		1			
	4		4.5			
	2		3			
	6.5		3			
			5			

Figure 1: Olfactory mucosal neural responses of stage 17 prolarvae following the application of one drop of test solutions.
A. 10^{-12} M petromyzonol sulfate; water conditioned by conspecifics (sibling familiar)
B. water conditioned by stage 12 eggs; water conditioned by ammocoetes

Figure 2: The response magnitude of olfactory mucosal responses from stage 17 prolarvae. The value for response magnitude is the average of the amplitude of the response (from table 1)/average response magnitude for 10^{-6} M L-arginine (from table 1). tca, taurocholic acid; petromz. sulf., petromyzonol sulfate; omega arg, Nω-nitro-L-arginine; NMA, N^G-methyl-L-arginine.

A



petromyzonol sulfate

prolarvae

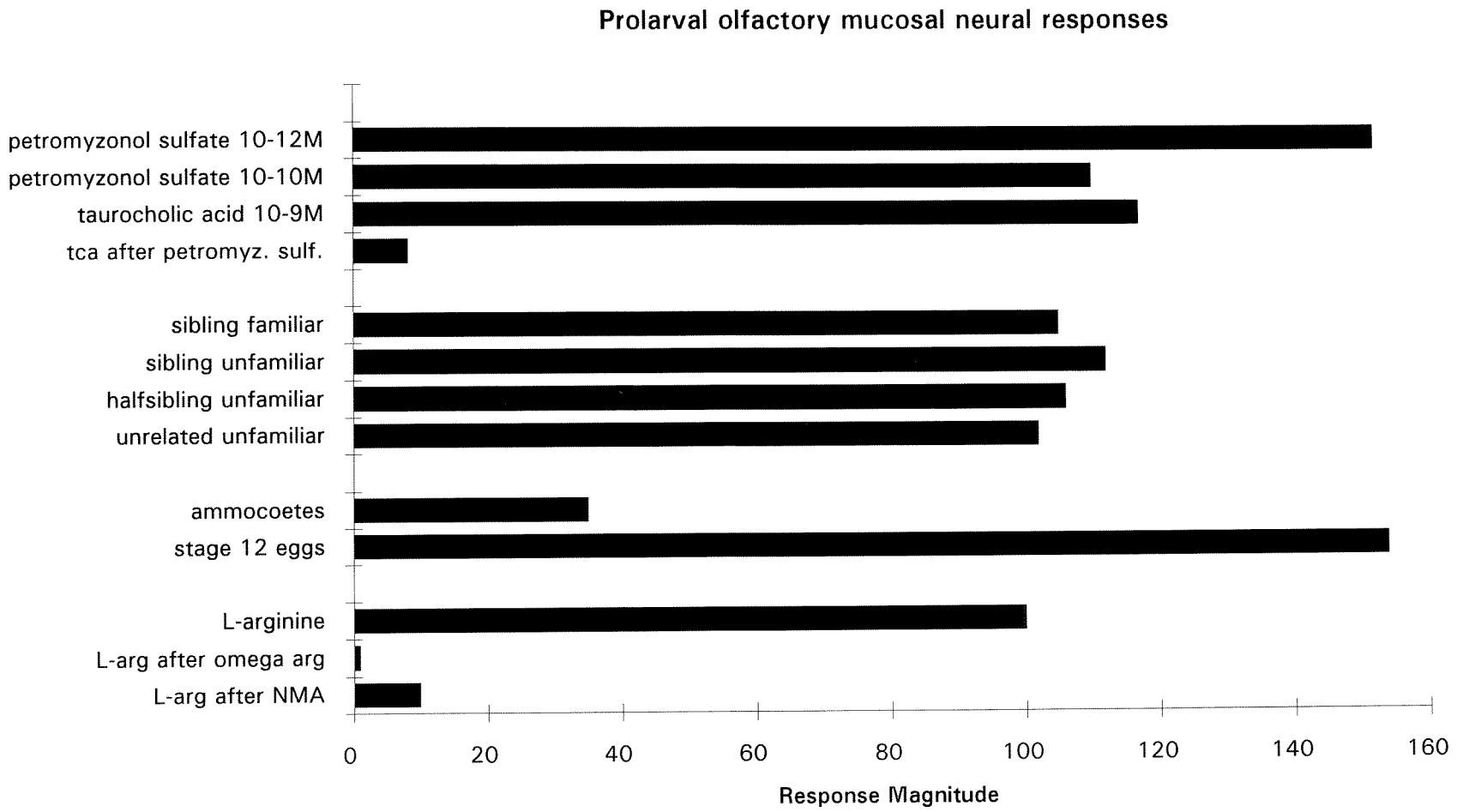
B

eggs

ammocoetes

Figure 1

Figure 2.


Prolarval olfactory mucosal neural responses
petromyzonol sulfate 10-12M
petromyzonol sulfate 10-10M
taurocholic acid 10-9M
tca after petromyz. sulf.
sibling familiar
sibling unfamiliar
halfsibling unfamiliar
unrelated unfamiliar
ammocoetes
stage 12 eggs
L-arginine
L-arg after omega arg
L-arg after NMA
0
20
40
60
80
100
120
140
160
Response Magnitude