Pharmacology Biochemistry & Behavior, Vol. 17, pp. 363–366, 1982. Printed in the U.S.A.

Schooling Behavior of Tadpoles: A Potential Indicator of Ototoxicity

ANDREW M. LUM,* RICHARD J. WASSERSUG,†[1]
MICHAEL J. POTEL‡ AND STEPHEN A. LERNER§

**John A. Burns School of Medicine, University of Hawaii, Honolulu, HI 96822*
†Department of Anatomy, Dalhousie University, Halifax, Nova Scotia, B3H 4H7, Canada
‡Department of Biophysics and Theoretical Biology, University of Chicago, Chicago, IL 60637
and
§Departments of Medicine and Microbiology, University of Chicago, Chicago, IL 60637

Received 12 February 1982

LUM, A. M., R. J. WASSERSUG, M. J. POTEL AND S. A. LERNER. *Schooling behavior of tadpoles: A potential indicator of ototoxicity* PHARMAC. BIOCHEM. BEHAV. 17(2) 363–366, 1982.—Fish and tadpoles in schools use hair cells of their lateral line system to assess their position in relation to neighbors. This suggests that pharmaceutical agents that damage hair cells in the mammalian inner ear may also alter geometry in fish and tadpole schools. We used a computer-based image analysis system to examine the effect of the ototoxic aminoglycoside antibiotic, streptomycin, on school geometry for tadpoles of the African clawed frog *Xenopus laevis*. Tadpoles exposed to streptomycin in the surrounding water show a general tendency toward clumping, and an increase in the distance over which they orient parallel to neighbors, compared to controls. These behavioral responses appear in 18 min or less, and are evident in some tadpoles exposed to concentrations as low as 5 μg/ml. Results suggest that analysis of spatial relations in tadpole schools could serve as a method for rapidly detecting ototoxic potential of agents suspected of damaging hair cells.

Aminoglycosides Hair cells Ototoxicity Schooling behavior Streptomycin Tadpoles *Xenopus laevis*

MANY drugs impair hearing and vestibular function in man and other mammals by damaging cochlear and vestibular hair cells [9]. The lateral line system of fish and aquatic Amphibia includes similar cells [6]. Wersäll and Flock [22] demonstrated that the microphonic output of the hair cells of the lateral line system in individual fish (*Lota vulgaris*) was inhibited by the presence of the ototoxic aminoglycoside antibiotic, streptomycin, in the water. In fish and amphibians hair cells are external and bathed by the surrounding water, whereas in mammals they lie in the inner ear accessible only to parenterally administered drugs which must penetrate from the vascular space [19, 5, 10]. The lateral line system is known to regulate the schooling behavior of certain fish [14,12]. Some species of tadpoles have well developed lateral lines and also form schools [20]. Given that the lateral line is a sensory mode used by tadpoles in regulating their schooling, as suggested in previous work [8,21], then the exposure of tadpoles to streptomycin should change their school geometry. The goal of the present study was to document changes in the geometric structure of tadpole aggregates as a result of exposure to concentrations of streptomycin in the same range as those present in the serum of patients treated with this drug. Our results lead to speculation about the possibility of using schooling behavior to assess pharmaceutical agents for ototoxicity.

METHOD

Tadpoles of the African clawed frog, *Xenopus laevis*, were used for this study because their lateral line system is well developed [18], and they form stable, stationary midwater schools with neighboring individuals generally oriented in the same direction [8,21].

Larvae from a single clutch of eggs were raised on a standard laboratory diet of baker's yeast under an approximately 13L:11D light regimen. Tadpoles grew normally and rapidly under these conditions. All larvae for experiments were between Gosner [7] developmental stages 25 and 34.

Two circular glass dishes (170 mm diameter×100 mm height) were used as test chambers. Each was positioned directly below a 35 mm camera fitted with a remote cable that could be operated by the experimenter outside the room. A photograph of a 10-cm ruler was taken with each camera to calibrate the size of tadpoles in later photographs. Ninety eight (±one) similarly sized tadpoles and 500 ml of stock tank water were placed in each dish. A bubble stone was added for aeration, and the larvae were given at least one hour to acclimate. All experiments were performed at the same time of day during the light phase of the tadpole's diel cycle. However, all photographs were taken with a flash in a completely darkened room to eliminate visually mediated behavior.

[1]Send reprint requests to R. J. Wassersug, Department of Anatomy, Dalhousie University, Halifax, Nova Scotia, B3H 4H7, Canada.

TABLE 1
EXPERIMENTAL TREATMENTS AND RESULTS

Experimental Groups*		Spatial Distribution		Interactive Distance In:		
Streptomycin Concentration (μg/ml)	Number of Photographs for Each Camera	Distance Between First Nearest Neighbors in cm (Mean (SD))	Morisita Index	Average number of nearest neighbors	cm	Body Lengths
0.0	5	0.80 (0.42)	0.99	5	2.11	4.5
151.0	5	0.75 (0.42)	1.04	11	3.25	7.1
0.0	5	0.81 (0.46)	1.03	6	2.34	4.4
44.0	5	0.77 (0.41)	1.04	27	5.69	11.2
0.0	5	0.81 (0.43)	1.00	6	2.31	4.6
13.5	5	0.74 (0.43)	1.04	6	2.28	4.8
0.0	5	0.75 (0.42)	1.01	3	1.56	3.1
5.0	5	0.71 (0.38)	1.11†	20	4.45	9.1
0.0	5	0.79 (0.44)	1.02	5	2.07	4.2
2.2	5	0.74 (0.42)	1.10†	5	2.10	4.2
0.0	3	0.70 (0.42)	1.00	5	2.09	4.5
0.0 (sham)	4	0.80 (0.43)	0.99	5	2.03	4.2
Overall controls:		0.79 (0.43)	1.01	5	2.07	4.2
Overall streptomycin:		0.74 (0.41)	1.07	14	3.55	7.3

*Each experimental treatment is preceded by its control. Streptomycin concentrations based on radioenzymatic assay (see text).
†Significant clumping at $p<0.01$.

Six experiments were performed, with tadpoles photographed after exposure to streptomycin at concentrations from 2.2 to 151 μg/ml. Before each photograph, the larvae were disturbed by removal of the bubble stone and by agitation with a net. This assured that tadpole positions were independent between sequential photographs so that photographic data could be pooled. After the disturbance, the experimenter left the room and turned off the lights; there was then a 3-min recovery period before additional pictures were taken. This is longer than necessary for *X. laevis* larvae in the dark to re-establish schools after disturbance [8]. After each photograph the bubble stone was replaced for 30 sec of aeration.

Control photographs were taken first, then the larvae were removed and a specified amount of streptomycin was added to the water. The water was mixed well to avoid concentration gradients, and the tadpoles were then returned to the tests chambers. The maximum amount of time between the first and last photograph for each drug treatment was 18 min. We carried out a sham experiment to determine whether our handling procedures or some extraneous variable associated with moving the tadpoles affected behavior. Each group of tadpoles was used at only one drug concentration.

After each experiment, an aliquot of water was removed from the chamber and its streptomycin concentration was assayed radioenzymatically [4] with ^{14}C-ATP (Amersham Corporation, final specific activity 8 Ci/mole) at a final concentration of 0.2 mM.

The photographs were analyzed at the University of Chicago with the Galatea image analysis system [15,16]. The size, position, and orientation of each tadpole in each photograph was digitized, stored and subsequently analyzed on the PDP-11 minicomputer of the Galatea system following procedures discussed in [8, 17, 21]. For each experiment and its control, the distance between all first nearest neighbors was calculated.

The Morisita Index [11] was used to assess spatial distribution. An index of 1.0 indicates a random distribution; >1 indicates a tendency toward clumping; and <1 implies an even distribution.

The greatest distance over which tadpoles orient significantly parallel to neighbors, i.e., their interactive distance was determined by techniques detailed in [8,21]. Those techniques yield an average value for interactive distance in terms of numbers of nearest neighbors. If the interactive distance is five, this means that there is a significant correlation between the compass direction of every tadpole and the five tadpoles nearest to it, but that there is no significant correlation between the orientation of each tadpole and others beyond its five most immediate neighbors. The interactive distance in nearest neighbors was converted to both actual distance in cm and to distances in relative units of average tadpole lengths for each experiment.

RESULTS

At all concentrations of streptomycin, tadpoles moved closer to their first nearest neighbor after exposure to the drug (Table 1, column 3). Surprisingly, only the two lowest con-

centrations were significantly clumped according to the Morisita Index ($p<0.01$).

Tadpoles exposed to streptomycin at 151, 44, and 5 μg/ml interacted (i.e., oriented parallel to each other) over much greater distances than controls. However, tadpoles exposed to streptomycin at 13.5 and 2.2 μg/ml did not demonstrate this increased parallel orientation. A Spearman Rank Correlation Test showed no significant correlation between streptomycin concentration and the average interactive distance ($r=0.5$; $p>0.20$). In the absence of a clear dose-response curve, the results for all controls and all treated tadpoles were pooled for further comparison. The average interactive distance for all controls extended to the fifth nearest neighbor, equivalent to a distance of 2.07 cm or 4.2 body lengths. In contrast, all streptomycin-treated tadpoles collectively showed an average interactive distance extending to the fourteenth nearest neighbor, over a distance of 3.55 cm or 7.3 body lengths (42.5% greater than the pooled controls).

Comparison of the sham-treated larvae and their controls reveal weak, shifts away from clumping and to shorter interactive distances, i.e., effects opposite to those found in all streptomycin-treated larvae versus their controls.

DISCUSSION

Streptomycin at a concentration of 44 μg/ml and apparently in certain circumstances as low as 5 μg/ml—concentrations readily achieved in the serum of patients treated with streptomycin—rapidly alters the geometry in schools of *Xenopus* larvae. This alteration occurs within 18 min of exposure and appears as a subtle shift from a random toward a clumped distribution with an increase in the distance over which tadpoles orient in parallel. The rapidity of the response is consistent with the hypothesis that superficial hair cells of the lateral line system are affected by the drug. Schooling fish similarly move closer to neighbors after surgical lesions of the acoustico-lateralis nerve [3,12].

The increase in interactive distance in treated tadpoles may seem unexpected, since these individuals should be less sensitive to their neighbors and, presumably, less interactive. There is, however, a partial explanation: when the sensitivity of the lateral line system is reduced the tadpoles are forced to move closed to one another to maintain their sense of spatial balance. Packing constraints then occur, secondarily forcing tadpoles to orient in parallel to avoid touching. This interpretation, however, explains neither the slight change in interactive distance at drug concentration 13.5 μg/ml compared to higher or lower concentrations, nor the significant Morista index in the absence of a major change in interactive distance at the lowest drug concentration. Clearly, additional data are necessary to resolve potential false negatives (or positives) in the data set.

Although we can not generate a simple dose-response curve from our data to date (nor was production of such a curve a goal of this preliminary study), a general effect of streptomycin on tadpoles is clear. The response pattern suggests that changes in tadpole schooling behavior could ultimately be used to detect toxicity of pharmaceutical agents suspected of affecting hair cells of the inner ear. Despite their taxonomic distance from man a potential advantage in using fish or tadpoles rather than mammals to assess toxic effects on hair cells is that with these lower vertebrates one can determine the exact concentration of the drug delivered to target cells without surgical manipulation or perturbation. The study of school geometry specifically allows one to examine the biological effect of a drug collectively on large numbers of animals. Both the electrophysiologic responses of the fish lateral line system [1] and the integrity of fish schools [2] have been proposed previously as bioassays for environmental pollutants. However, to our knowledge neither has been developed into a practical assay. Because *Xenopus* larvae form stationary rather than moving schools, slight shifts in their schooling geometry are inherently easier to document than changes in school structure for swimming fish.

ACKNOWLEDGEMENTS

This research was supported by an Edmundson Fellowship and a Loeb Scholarship from the University of Chicago to AML; a Spencer Fund Grant and a Dalhousie Faculty of Medicine Research Grant to RJW; the Louis Block Fund of the University of Chicago and NIH grant RR-05367 to MJP; and by the Infectious Disease Research Fund of the University of Chicago to SAL. F. Breden, M. McClintock, D. Nance and L. Won, offered helpful comments on the manuscript.

REFERENCES

1. Bahr, T. G. Electrophysiological responses of trout to dissolved oxygen and cyanide. In: *Bioassay Techniques and Environmental Chemistry*, edited by G. E. Glass. Ann Arbor: Ann Arbor Science Publishers Inc., 1973, pp. 231–255.
2. Besch, W. K., A. Kembell, K. Meyer-Waarden and B. Scharf. A biological monitoring system employing rheotaxis of fish. In: *Biological Monitoring of Water and Effluent Quality*, edited by J. Cairns, Jr., K. L. Dickson and G. F. Westlake. Philadelphia: American Society for Testing and Materials, Special Technical Publ. 607, 1976, pp. 56–74.
3. Burgess, J. W. and E. Shaw. Effects of acoustico-lateralis denervation in a facultative schooling fish: a nearest-neighbor matrix analysis. *Behav. Neural Biol.* **33:** 488–497, 1981.
4. Dickie, P., L. E. Bryan and M. A. Pickard. Effect of enzymatic adenylylation on dihydrostreptomycin accumulation in *Escherichia coli* carrying an R-Factor; Model explaining aminoglyoside resistance by inactivating mechanisms. *Antimicrob. Agents Chemother.* **14:** 569–580, 1978.
5. Federspil, R. Pharmacokinetics of aminoglycoside antibiotics in the perilymph. In: *Aminoglycoside Ototoxicity*, edited by S. A. Lerner, G. J. Matz and J. E. Hawkins. Boston: Little, Brown and Co., 1981, pp. 99–108.
6. Flock, A. Ultrastructure and Function in the lateral line organs. In: *Lateral Line Detectors*, edited by P. H. Cahn. Bloomington: Indiana University Press, 1967, pp. 163–197.
7. Gosner, K. L. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* **16:** 183–190, 1960.
8. Katz, L. C., M. J. Potel and R. J. Wassersug. Structure and mechanisms of schooling in tadpoles of the clawed frog, *Xenopus laevis*. *Anim. Behav.* **29:** 20–33, 1981.
9. Lerner, S. A., G. J. Matz and J. E. Hawkins. *Aminoglycoside Ototoxicity*. Boston: Little, Brown and Co., 1981.
10. Lerner, S. A., R. Seligsohn, I. Bhattacharya, R. Hinojosa and G. J. Matz. Pharmacokinetics of gentamicin in the inner ear perilymph of man. In: *Aminoglycoside Ototoxicity*, edited by S. A. Lerner, G. J. Matz and J. E. Hawkins. Boston: Little, Brown and Co., 1981, pp. 357–369.
11. Morisita, M. Estimation of population density by spacing method. *Mems Fac. Sci. Kyushu Univ* Series E (Biol.) **1:** 187–197, 1954.
12. Partridge, B. L. and T. Pitcher. The sensory basis of fish schools: relative roles of lateral line and vision. *J. comp. Physiol.* **35:** 315–325, 1980.

13. Partridge, B. L., T. Pitcher, J. M. Cullen and J. Wilson. The three-dimensional structure of fish schools. *Behav. Ecol. Sociobiol.* **6:** 277–288, 1980.
14. Pitcher, T., B. L. Partridge and C. S. Wardle. A blind fish can school. *Science* **194:** 963–965, 1976.
15. Potel, M. J., R. E. Sayre and A. Robertson. A system for interactive film analysis. *Comput. Biol. Med.* **9:** 237–256, 1979.
16. Potel, M. J., R. E. Sayre and S. A. MacKay. Graphics input tools for interactive motion analysis. *Comput. Graph. Image Process.* **14:** 1–23, 1980.
17. Potel, M. J. and R. J. Wassersug. Computer tools for the analysis of schooling. *Envir. Biol. Fishes* **6:** 15–19, 1981.
18. Shelton, P. M. The lateral line system at metamorphosis in *Xenopus laevis* (Daudin). *J. Embryol. exp. Morphol.* **24:** 511–524, 1970.
19. Tran Ba Huy, P., C. Manuel and A. Meulemans. Kinetics of aminoglycoside antibiotics in perilymph and endolymph in animals. In: *Aminoglycoside Ototoxicity*, edited by S. A. Lerner, G. J. Matz and J. E. Hawkins. Boston: Little, Brown and Co., 1981, pp. 81–97.
20. Wassersug, R. J. Aspects of social behavior in anuran larvae. In: *Evolutionary Biology of the Anurans: Contemporary Research on Major Problems*, edited by J. L. Vial. Columbia: University of Missouri Press, 1973, pp. 273–297.
21. Wassersug, R. J., A. M. Lum and M. J. Potel. An analysis of school structure for tadpoles (Anura: Amphibia). *Behav. Ecol. Sociobiol.* **9:** 15–22, 1981.
22. Wersäll, J. and A. Flock. Suppression and restoration of the microphonic output from the lateral line organ after local application of streptomycin. *Life Sci.* **3:** 1151–1155, 1964.