6 Stamatis, N., Yannopoulos, G., and Pelecanos, M., Genet. Res. *38* (1981) 125.
7 Yannopoulos, G., Stamatis, N., Zacharopoulou, A., and Pelecanos, M., Mutat. Res. *83* (1981) 383.
8 Yannopoulos, G., Genet. Res. *32* (1978) 239.
9 Lindsley, D. L., and Grell, E. H., Carnegie Institute of Washington, Publ. No. 627 (1968).
10 Iwano, M., Kattor, K., Saigo, K., Matsuo, Y., Mukai, T., and Yamazaki, T., Jap. J. Genet. *59*(4) (1984) 403.
11 Yannopoulos, G., and Pelecanos, M., Genet. Res. *29* (1977) 231.
12 Yannopoulos, G., Stamatis, N., and Zacharopoulou, A., Heredity *49*(2) (1982) 235.

0014-4754/86/11/121283-03$1.50 + 0.20/0

Thalamic stimulation evokes sex-color change and gamete release in a vertebrate hermaphrodite

L. S. Demski and J. G. Dulka

School of Biological Sciences, University of Kentucky, 101 Morgan Bldg, Lexington (Kentucky 40506, USA), 26 February 1986

Summary. Sperm and egg release and sex-color patterns specific for the male and female phases of reproductive behavior were elicited by electrical stimulation in the thalamus of anesthetized sea bass. Thalamic switching of the sex-role specific motor activities in response to visual signals from the mate is considered an important feature regulating the complex mating activity of these simultaneous hermaphrodites.

Key words. Serranus subligarius; color change; sexual behavior; sperm and egg release; hermaphroditism; thalamus; brain stimulation; hormones and behavior; visual signals; fishes.

Interactions between brain systems for male versus female behavior must occur in hermaphrodites which simultaneously produce both eggs and sperm but normally express only one sex phase at any given time[1,2]. We have studied neural mechanisms for sex-color change and gamete release in one of these unique animals as a means of better understanding such interactions and hence the sensorimotor control of vertebrate reproductive behavior in general.

Serranus subligarius is a small sea bass plentiful along the southern Atlantic and Gulf coasts of the United States. During its daily spawning period, an individual will typically mate as both female and male, with a pair switching roles several times[2-4]. Initially, the fish are noticeably bloated with eggs and exhibit a *dark posterior* (DP) color pattern associated with female behavior (fig. 1B). Following vigorous courtship, the more submissive animal begins to display male-typical behavior and a *banded* (Bd) coloration (fig. 1A). At the height of courting, the male-phase animal is strongly Bd and the individual in the female role retains the DP. As spawning approaches, a normally dark spot on the dorsal fin in the female-phase animal begins to fade. The lightening can also extend down to the side of the body below the spot. The spawning is initiated when the female takes a head-up posture, quivers and flashes a *reverse V* (RV) color pattern that is the complement of the Bd (fig. 1C). The pair then dart toward the surface and perform the spawning act or snap with the 'female' curved in front of the 'male'.

The sex-color patterns of *S. subligarius* are useful for the analysis of neural systems controlling rapid sex change since they are indicators of the sex phases and can be studied in anesthetized-immobilized preparations suitable for neurophysiology. In this project, we mapped brain areas from which the three prominent sex-color patterns of *S. subligarius* (fig. 1 A–C) as well as gamete release can be evoked by electrical stimulation. Experimental procedures were adapted from earlier studies in freshwater fishes[5,6].

Color patterns and gamete release triggered by thalamic stimulation

		Evoked response thresholds				
Site	Fish No.	Bd	DP	RV	SR	ER
1	8	50 µA	—	—	—	—
2	15	60 µA	—	(60 µA)	—	—
3	3	100 µA	—	—	—	—
4	16	—	80 µA	—	—	80 µA
5	7	—	50 µA	(50 µA)	20–50 µA	—
6	15	50 µA	—	—	—	—
7	14	50 µA	—	—	—	—
8	12	80 µA	—	20 µA; (50 µA)*	50–100µA	—

Bd, banding; DP, dark posterior; ER, egg release; RV, reverse V; SR, sperm release; ( ), after-response at termination of stimulation: *weak responses were evoked during 20 µA stimulation while strong after-response occurred following some of the tests of 50 µA.

Fish were captured during July and August using SCUBA at St. Andrews State Park, Florida. With the exception of one animal (see below), they were transported to the Gulf Coast Research Laboratory, Ocean Springs, MS, and maintained in stock tanks. Most fish were tested within 1 week of capture while still spermiated but not before they stopped ovulating which occurred after 1 or 2 days. Our data on female color patterns and egg release are thus limited. Fish were anesthetized in 2% urethane and then placed in a surgical holder and perfused through the mouth with seawater (fig. 1D). Evoked color changes were videotaped for later analysis and gamete release was monitored by placing a small container of seawater under the fish's abdomen (fig. 1E). Electrodes were either tapered stainless steel insect pins or commercially available microelectrodes. Stimulation was provided by a Grass S88 stimulator and PSIU-4 constant current converter and consisted of 50 Hz, 1-ms square-waves with currents up to 100 µA. Electrodes were lowered from the dorsal surface of the exposed brain with current set at 100 µA. When a positive response occurred, the current was reduced and the electrode moved up and down to find the most sensitive area. Evoked responses were repeatable (at least 5 × consecutively) with similar latencies (approximately 10–30 s). Areas of interest were marked by iron deposition for identification using the Prussian blue reaction with a neutral red counterstain.

An attempt was made to sample all major areas of the brain. Sex-related color changes were elicited on 48 electrode tracks in 25 animals and 15 sites in 10 fish were identified. Many other tracks were negative. This paper deals specificially with 8 sites clustered in the thalamus, the only area from which all three color patterns were evoked (fig. 2). Other sites positive for 1 or 2 of the responses were located in the area ventralis telencephali pars supracommissuralis, the preoptic area and adjacent optic nerves, the optic nerve and adjacent pretectum-thalamus, the torus semicircularis and ventral tegmentum of the midbrain and medial reticular formation of the medulla. The latter results have been described elsewhere[2,7].

As mentioned, each of the three color patterns was evoked by thalamic stimulation; in most cases more than one response was

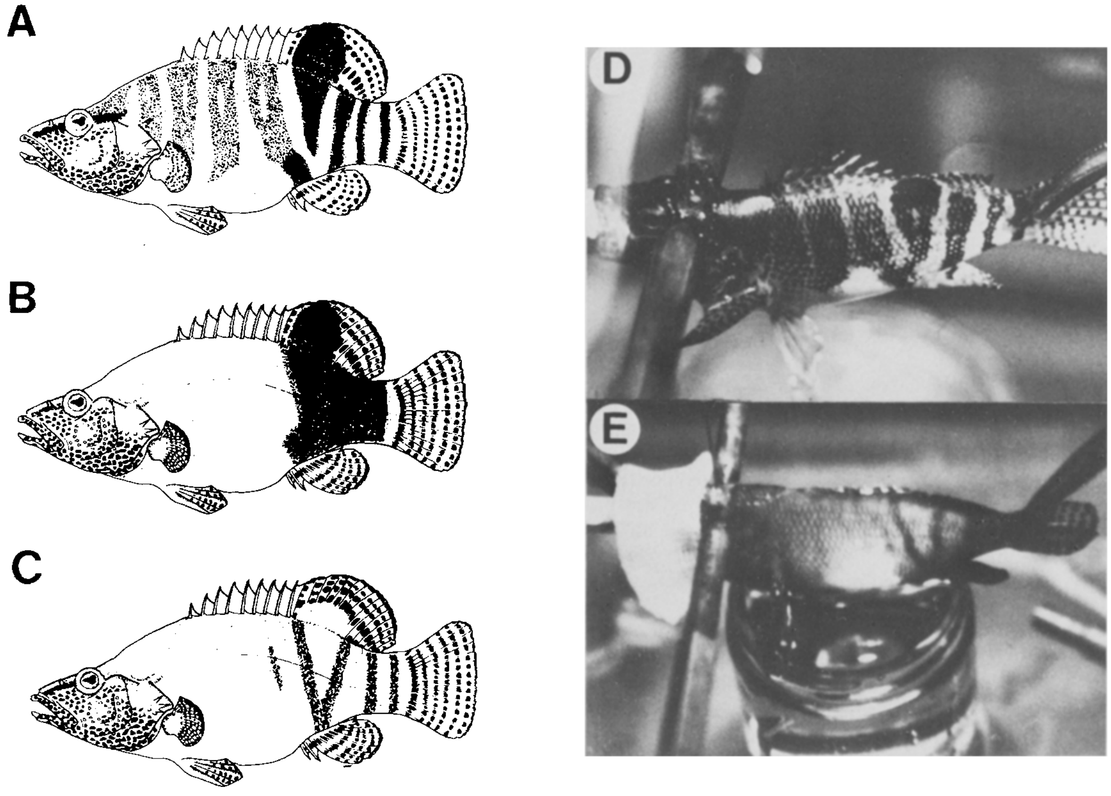

Figure 1. Left side: Drawings of the three major color phases of *Serranus subligarius*. The figures were made from courtship and spawning activity videotaped in the field. *A* The *banded* pattern (Bd) is typical of male-phase animals. Bands are always darker on the posterior half of the fish. Sperm can usually be expressed from fish showing this pattern. *B* The *dark posterior* pattern (DP) is typical of female-phase animals during courtship. These fish are usually bloated with freshly ovulated eggs. *C* The *reverse V* (RV) is a color phase observed in 'females' just prior to the spawning climax or snap. Note that the RV is the partial complement of the Bd phase. Right side: Color phase responses evoked by electrical stimulation of the brain: intense Bd (D) and RV (E). Both fish were slightly Bd prior to stimulation and returned to this pattern within several min following a test. Gamete discharge was monitored by placing the urogenital openings into a small container of seawater (seen in E).

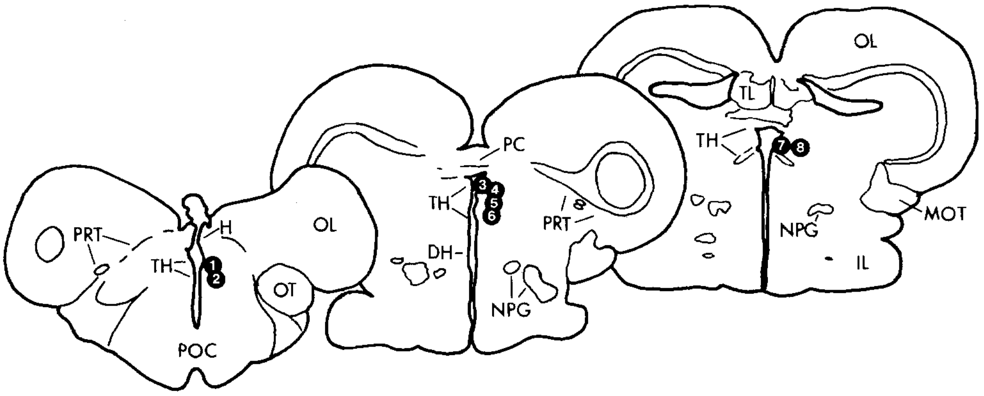

Figure 2. Histological identification of thalamic stimulation sites based on Prussian blue staining. Eight points from which sex-color changes were evoked at thresholds at or below 100 µA are represented. See text for details of the responses and stimulation parameters used. The points are plotted on representative transverse sections of the diencephalon. Nomenclature is based primarily on Braford and Northcutt[8]. Sites originally on the right side have been transposed to the left in the diagram. Abbreviations: DH, dorsal hypothalamus; H, habenula; IL, inferior lobe of hypothalamus; MOT, marginal branch of the optic tract; NPG, nucleus preglomerulosus; OL, optic lobe; OT, optic tract; PC, posterior commissure; POC, postoptic commissures; PRT, pretectal area; TH, thalamus; TL, torus longitudinalis.

associated with a single point (see table for details, e.g., thresholds). Bd was evoked from sites 1–3 and 6-8. A strong response is illustrated in figure 1D. The activity occurred within s of the stimulation and could last for several s following a test. DP resulted from stimulation of sites 4 and 5. In several cases, Bd or DP occurred during the stimulation and RV followed as an after-response at its offset (sites 2, 5, 8; fig. 1E). The RV was also observed during stimulation of site 8.

Sperm release was triggered during stimulation of sites 5 and 8 while egg discharge occurred during stimulation of site 4. In the latter case, the fish was captured at the beginning of the daily spawning period and was bloated with eggs. The animal was tested at our temporary campsite laboratory within several hours.

Although widespread brain regions were stimulated, gamete release and all three color phases were evoked only from the thalamus. The findings strongly suggest that the area controls reproductive activity and may be an important locus for interactions between male and female systems. Studies in other fishes are consistent with the present results in that stimulation of specific thalamic sites elicited sperm release in goldfish and sunfish and light organ discharge, a courtship display, in midshipmen[6].

The thalamus of teleosts is known to receive input from both the retina[8–11] and optic tectum[8] and thus presumably integrates visual information. The anatomy is consistent with a thalamic control of sex-color phases in response to the visual displays of other individuals. In this regard, the brain stimulation could be activating either thalamic afferents or efferents or both simultaneously. Cases where one pattern was evoked during stimulation and another as an after-response could be due to activation of systems which inhibit other systems during stimulation. The after-response is observed as the latter rebound to the inhibition. Confirmation of such mechanisms must await neurophysiological analysis and the relationship of such after-responses to natural behavior is also unknown.

Reproductive activity in sea bass is seasonal and the DP and RV patterns have only been observed during the spawning period[2–4]. The thalamus of some teleosts contains sex-steroid concentrating neurons[12] and gonadotropin-releasing hormone (GnRH)-containing cell bodies and/or axons[13,14]. If such hormone-specific neuronal distributions are also present in sea bass, they could provide substrates for seasonal control of thalamic sensitivity and hence sexual coloration.

Sex-steroid concentrating neurons are also present in the thalamus of jawless fishes, amphibians, reptiles, birds and mammals while GnRH-containing cells or fibers are reported in the area in amphibians and mammals[15]. Electrical stimulation of the caudal intralaminar thalamus in monkeys evokes penile erection and genital scratching[16]. The comparative data extend our hypothesis of sex-hormone modulation of thalamic sensorimotor pathways to mammals and other vertebrates. Such thalamic mechanisms may indeed be primitive features underlying the evolution of the diverse and highly successful reproductive behavior characteristic of vertebrates[17].

Acknowledgments. We thank M.B. Demski, H.E. Sloan and P.J. Hornby for technical assistance and suggestions on the manuscript. Use of facilities at the Gulf Coast Research Laboratory and St. Andrews State Park are also greatly appreciated. Supported by BioMedical Research Funds, a grant from the University of Kentucky Research Foundation and NIH grant NS 19431.

1 Fischer, E.A., Am. Nat. *117* (1981) 64.

2 Demski, L.S., in: Psychobiology of Reproductive Behavior: An Evolutionary Perspective. Ed. D. Crews. Prentice-Hall, Englewood, NJ 1986, in press.

3 Clark, E., Science *129* (1959) 215.

4 Demski, L.S., and Dulka, J.G., Am.Zool. *23* (1984) 881.

5 Bauer, D.H., and Demski, L.S., J. exp. Biol. *84* (1980) 149.

6 Demski, L.S., in: Fish Neurobiology, vol. 2, p. 317. Eds R.E. Davis and R.G. Northcutt. Univ. of Michigan Press, Ann Arbor 1983.

7 Demski, L.S., Dulka, J.G., and Hornby, P.J., Neurosci. Abstr. 122.10 (1984) 404.

8 Braford, M.R. Jr, and Northcutt, R.G., in: Fish Neurobiology, vol. 2, p. 117. Eds R.E. Davis and R.G. Northcutt. Univ. of Michigan Press, Ann Arbor 1983.

9 Fraley, S.M., and Sharma, S.C., Cell Tiss. Res. *238* (1984) 529.

10 Presson, J., Fernald, R.D., and Max, M., J. comp. Neurol. *235* (1985) 360.

11 Springer, A., and Mednick, A.S., J. comp. Neurol. *236* (1985) 179.

12 Demski, L.S., and Hornby, P.J., Can. J. Fish aquat. Sci. *39* (1982) 36.

13 Münz, H., Stumpf, W.E., and Jennes, L., Brain Res. *221* (1981) 1.

14 Borg, B., Goos, H.J.Th., and Terlou, M., Cell Tiss. Res. *226* (1982) 695.

15 Demski, L.S., Am. Zool. *24* (1984) 809.

16 MacLean, P.D., Dua, S., and Denniston, R.H., Archs Neurol. *9* (1963) 485.

17 Crews, D., BioScience *33* (1983) 545.

0014-4754/86/11/121285-03$1.50 + 0.20/0

Adaptive optimal sound for vocal communication in tunnels of a subterranean mammal *(Spalax ehrenbergi)*[1]

G. Heth, E. Frankenberg* and E. Nevo

*Institute of Evolution, University of Haifa, Mt. Carmel, Haifa 31999 (Israel), and *Nature Reserves Authority, 78 Yirmeyahu St., Jerusalem 94467 (Israel), 12 December 1985*

Summary. The adaptive value of sound signal characteristics for transmission in the underground tunnel ecotope was tested using tunnels of the solitary territorial subterranean mole rats. We analyzed the propagation of synthetic calls with various frequencies through natural tunnels along different distances. Here we present evidence that sound propagation proved efficient only across short distances (a few meters). The least attenuation of sounds occurred at low frequencies. The 440 Hz sound was transmitted better than the lower (220 Hz) or higher (880, 1760, 3520 Hz) tested frequencies. These characteristics matched perfectly with the mole rat features of vocalization and hearing, thus reflecting the operation of natural selection for adaptive vocal communication in the underground tunnel ecotope.

Key words. Sound transmission underground; vocal communication; subterranean mole rats.

Subterranean rodents exhibit varied degrees of atrophy of eyes and vision and hypertrophy of olfaction and vocal communication[2–6]. Auditory communication is important for existence in the unique underground ecotope and serves a wide range of functions, such as aggression, territoriality[7,8], assortative mating and ethological reproductive isolation[9–11]. The study of vocal communication of subterranean rodents has been conducted to date only in few genera, *Spalacopus*[12], *Talpa*[6,13,14] and *Spalax*[7,11,15], and should be extended. Courtship calls of mole rats *Spalax ehrenbergi* were analyzed recently[15]; they ranged primarily from 500 to 4500 Hz, peaking at around 500 Hz. A study of cochlear microphonics of mole rats (manuscript in preparation) matched the above vocalization data very well. The frequency range was found to be between 0.1 and 8 kHz, with a sensitivity maximum between 0.5 and 1.0 kHz. In addition, recordings of evoked potentials from the inferior colliculus of the mole rat midbrain revealed a sensitivity maximum at around 0.5 kHz. Studies of vocalizations in *Spalacopus*[12], *Talpa*[6,13] and *Spalax*[7,11,15] revealed also low frequency calls. It seems therefore that low frequency calls may characterize both subterranean insectivores and rodents. The adaptive value of calls in a certain habitat should be tested according to their maximal efficiency in