ization (*23*). The observations reported here, taken with earlier results, support the hypothesis that in immature animals, thyroid hormones and NGF interact at the cellular level to promote microtubule formation, axonogenesis, and possibly synaptogenesis (*7, 23, 25*). In the adult animal, such an interaction may be of importance for synaptic transmission and translocation of molecules across the cell membrane, possibly by the induction of a phosphatidylinositol effect (*26*) at the cell membrane.

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References and Notes

1. R. Levi-Montalcini, in *The Nature of Biological Diversity*, J. M. Allen, Ed. (McGraw-Hill, New York, 1963), p. 261; *Harvey Lect.* **60**, 217 (1966); ——— and P. U. Angeletti, *Physiol. Rev.* **48**, 534 (1968); B. Bjerre, A. Bjorklund, W. Mobley, *Z. Zellforsch. Mikrosk. Anat.* **146**, 15 (1973); P. U. Angeletti, R. Levi-Montalcini, F. Carmia, *Brain Res.* **27**, 343 (1971); B. Bjerre, L. Wiklund, D. L. Edwards, *ibid.* **92**, 257 (1975).
2. D. G. Johnson, P. Gorden, I. J. Kopin, *J. Neurochem.* **18**, 2355 (1971); I. A. Hendry, *Biochem. J.* **128**, 1265 (1972); H. D. Shine and J. R. Perez-Polo, *J. Neurochem.* **27**, 1315 (1976).
3. W. A. Frazier, L. F. Boyd, M. W. Pulliam, A. Szutowicz, R. A. Bradshaw, *J. Biol. Chem.* **249**, 5918 (1974).
4. W. J. Freed, *Brain Res. Bull.* **1**, 393 (1976).
5. P. Walker, M. E. Weichsel, Jr., S. M. Guo, D. A. Fisher, D. A. Fisher, in preparation.
6. J. A. Brasel and D. B. Boyd, in *Perinatal Thyroid Physiology and Disease*, D. A. Fisher and G. N. Burrow, Eds. (Raven, New York, 1975), p. 59; J. T. Eayrs and G. Horn, *Anat. Rec.* **121**, 53 (1955); S. E. Geel and P. S. Timiras, *Brain Res.* **4**, 135 (1967); J. L. Nicholson and J. Altman, *ibid.* **44**, 13 (1972); *ibid.*, p. 25; M. E. Weichsel, Jr., *ibid.* **78**, 455 (1974).
7. J. M. Lauder, *Brain Res.* **142**, 25 (1978).
8. W. C. Mobley, A. Schenker, E. M. Shooter, *Biochemistry* **15**, 5543 (1976).
9. The substances tested for immunological cross-reactivity that produced no displacement at a maximum concentration of 10 μg/ml were thyrotropin-releasing hormone; gonadotropin-releasing hormone; somatotropin release inhibiting factor; rat, ovine, and human thyroid-stimulating hormone; rat luteinizing, follicle-stimulating, and growth hormones; rat prolactin; synthetic adrenocorticotropic hormone (1-24); alpha melanocyte-stimulating hormone; porcine and bovine insulin; glucagon; pentagastrin; thymosin; liver cell growth factor; and human NGF. Human β-NGF was isolated from term human placenta [L. D. Goldstein, C. P. Reynolds, J. R. Perez-Polo, *Neurochem. Res.* **3**, 175 (1978)] and purified according to the method of Mobley *et al.* (*8*); 2 mg of human β-NGF was recovered with a biological activity of 10 ng per unit.
10. O. H. Lowry, N. J. Rosebrough, A. L. Farr, R. J. Randall, *J. Biol. Chem.* **193**, 265 (1951).
11. G. W. Snedecor, *Statistical Methods* (Iowa State College Press, Ames, ed. 5, 1956).
12. R. H. Angeletti, P. U. Angeletti, R. Levi-Montalcini, *Brain Res.* **46**, 421 (1972).
13. P. Walker and J. H. Dussault, in preparation.
14. D. N. Ishii and E. M. Shooter, *J. Neurochem.* **25**, 843 (1975).
15. B. Bjerre, A. Bjorklund, U. Stenevi, *Brain Res.* **60**, 161 (1973); *ibid.* **74**, 1 (1974).
16. J. E. Turner and K. A. Glaze, *Exp. Neurol.* **57**, 687 (1977); K. A. Glaze and J. E. Turner, *ibid.* **58**, 500 (1978).
17. B. D. Berger, C. D. Wise, L. Stein, *Science* **180**, 506 (1973).
18. L. E. Braverman, S. H. Ingbar, K. Sterling, *J. Clin. Invest.* **49**, 855 (1970); A. Fertig, J. A. Kiernan, S. S. Seyan, *Exp. Neurol.* **33**, 372 (1971); L. Guth, *ibid.* **45**, 606 (1974); E. Heinicke, *J. Neurol. Sci.* **31**, 293 (1977).
19. M. B. Dratman, *J. Theor. Biol.* **46**, 255 (1974); ———, F. L. Crutchfield, J. Axelrod, R. W. Colburn, N. Thoa, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 941 (1970).
20. J. R. Tata and C. C. Widnell, *Biochem. J.* **98**, 604 (1966); J. H. Oppenheimer, D. Koerner, H. L. Schwartz, M. I. Surks, *J. Clin. Endocrinol. Metab.* **35**, 330 (1972); A. Viarengo, A. Zoncheddu, M. Taningher, M. Orunesu, *Endocrinology* **97**, 955 (1975); W. H. Dillman, J. Mendecki, D. Koerner, H. L. Schwartz, J. H. Oppenheimer, *ibid.* **102**, 568 (1978); J. Bernal, A. H. Coleoni, L. J. DeGroot, *ibid.*, p. 452; S. Schapiro and C. J. Percin, *ibid.* **7**, 1075 (1966); S. J. Jothy, L. Bilodeau, H. Champsaur, H. Simpkins, *Biochem. J.* **150**, 133 (1975); D. T. Kurtz, A. E. Sippel, P. Feigelson, *Biochemistry* **15**, 1031 (1976).
21. I. A. Hendry, K. Stoeckel, H. Thoenen, L. L. Iversen, *Brain Res.* **68**, 103 (1974); K. Stoeckel and H. Thoenen, *ibid.* **85**, 337 (1975).
22. M. E. Schwab, *ibid.* **130**, 190 (1977).
23. R. Levi-Montalcini, R. Revoltella, P. Calissano, *Recent Prog. Horm. Res.* **38**, 635 (1974); P. Calissano and C. Cozzari, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2131 (1974).
24. J. Francon, A. Fellous, A-M.Lennon, J. Nunez, *Nature (London)* **266**, 188 (1977).
25. A. Szutowicz, W. A. Frazier, R. A. Bradshaw, *J. Biol. Chem.* **251**, 152A (1976).
26. J. Lakshmanan, *Biochem. Biophys. Res. Comm.* **82**, 767 (1978); R. H. Michell, *Biochim. Biophys. Acta* **415**, 8 (1975).
27. K. W. Jacobs, *Educ. Psychol. Meas.* **36**, 899 (1976).
28. Supported by PHS grants HD 11303, RR 05551, and HD 04270. P.W. was a Research Fellow of the Conseil de la Recherche en Santé du Québec. We thank R. T. Rubin, M. L. Weil, R.S. Swerdloff, A. R. Wolfsen, and H. V. Firemark for their critical review of the manuscript and R. T. Rubin for his expert assistance in statistical analysis of the data.

22 November 1978; revised 29 January 1979

Temporal Pattern as a Cue for Species-Specific Calling Song Recognition in Crickets

Abstract. *Female crickets can recognize conspecific calling song from its temporal pattern alone. In* Teleogryllus oceanicus, *the song pattern consists of three classes of interpulse intervals arranged in a stereotyped sequence. Females recognize a model song in which the sequential order of intervals is random. This argues against the hypothesis that recognition results from matching auditory input to an internal template of the song.*

Many animals can recognize species-specific signals (*1*). A current problem in neuroethology concerns the mechanisms underlying such recognition. One class of possible mechanisms entails comparison of sensory input with an internal model of the signal. For example, birds have been suggested to judge auditory input against a species-specific song blueprint or template, both to select a conspecific song model to imitate (*2*) and to guide the development of song motor patterns (*3*). Comparison of auditory input with an internal template has also been suggested as underlying species-specific phonotaxis in crickets (*4, 5*). Male crickets produce a species-specific calling song, which attracts conspecific females (*6*). The song consists of a series of sound pulses delivered according to a stereotyped temporal pattern generated by the central nervous system (*7*). Females can recognize their conspecific song on the basis of its temporal pattern (*8, 9*).

It has been suggested that the neural machinery constituting the hypothesized template for song recognition in females has some elements in common with the male's song-pattern generator (*5, 10*). Such an overlap in the neural substrates for song production and song recognition could explain two important observations. (i) The song pattern produced by males and the pattern preferred by females are genetically coupled; interspecific hybrid males have songs that differ from either of the parental types, and hybrid females prefer these hybrid songs (*5*). (ii) Song production and song recognition vary in similar ways with temperature; females prefer the songs of males that are singing at the same temperature at which they are listening (*8*). An extreme version of the hypothesis of overlap between the neural substrates for song production and recognition is that the template in the female (who does not sing) consists of an internal copy of calling song, produced by the same neural machinery responsible for song pattern production in the male. We now present evidence that argues against this extreme view.

The calling song of *Teleogryllus oceanicus* consists of a series of sound pulses separated by three distinct classes of interpulse intervals, arranged in a repeating stereotyped sequence (Fig. 1, A, B, and E). The repeating unit (phrase) of the song (Fig. 1A) can be described as a string of four intrachirp intervals followed by nine pairs of alternating inter- and intratrill intervals, followed by an additional intertrill interval, all of the intervals being separated by sound pulses (*11*). We reasoned that if female *T. oceanicus* recognize their species calling

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song by matching it against an internal model of the song, then this match would be destroyed if the three types of intervals, instead of occurring in their normal sequence, occurred in random order. We produced such a song by programming a computer to repeatedly shuffle the ordinal positions of the intervals of the normal *T. oceanicus* calling song phrase (*12*). The computer produced a series of electrical pulses in the required temporal sequence, and these pulses were used to trigger an electronic circuit, which synthesized the actual sound pulses (*13*) (Fig. 1C). The shuffled song (Fig. 1I) has interpulse intervals of the same durations and in the same relative numbers as normal song (Fig. 1H), but these intervals do not occur in any regular pattern (compare Fig. 1F with Fig. 1E).

We compared the attractiveness to *T. oceanicus* females of shuffled song, normal *T. oceanicus* song, and the song of a related species, *Teleogryllus commodus* (Fig. 1, D and G), in two behavioral assays. In a walking phonotaxis assay, a virgin female *T. oceanicus* was placed at the starting point in an arena in which two loudspeakers played two different songs (*14*). To minimize visual cues the speakers were hidden behind a gauze curtain. The cricket was allowed 3 minutes in which to leave the starting area and an additional 3 minutes to walk to and climb up the gauze curtain in front of one of the speakers. If she failed to meet either of the time limits or if she climbed up the curtain at a point not directly in front of one of the speakers, the trial was discarded. Each cricket was used only once. The relative numbers of crickets choosing each song indicated the relative attractiveness of the two songs of a pair. Positional effects were eliminated by alternating the speakers from which the two songs were played from trial to trial.

The second behavioral assay exploits the fact that tethered crickets in stationary flight in a windstream attempt to turn in response to sound stimuli (*15*). A component of their steering behavior is a rudder-like movement of the abdomen and hind legs in the direction of the attempted turn (Fig. 2D). In the discrimination assay, two songs were presented simultaneously from speakers located 90° to the cricket's left and right. An image of the cricket was displayed on a video monitor, and steering movements were observed both visually and electronically by a photocell mounted on the video monitor screen. The photocell was arranged so that, as the dark image of the abdomen and legs moved across the otherwise bright screen, the amount of light falling on the photocell varied. Thus the amplitude of the photocell output reflected the instantaneous position of the abdomen and legs. We presented the two songs first, for example, with song A from the left and song B from the right, and then reversed this relationship at 30-second intervals for a total of 3 minutes. The cricket responded to these reversals by moving its abdomen and legs to follow the preferred song; these movements are easily seen on the photocell records (Fig. 2, E-G).

In both the walking and flight assays the intensities and carrier frequencies of the two songs of a pair were carefully matched [intensities were 65 dB (measured at the starting point) in the walking assay and 75 dB (at the position of the cricket) in the flight assay (*16*); carrier frequencies were either 4.5 or 5 kHz in both assays]. Both assays took place in an anechoic room, with temperature held at 24° to 26°C. This temperature range is similar to that at which the natural calling songs upon which the synthetic models are based were recorded (23.5° to 25.5°C) (*11*).

To verify that *T. oceanicus* females could make species-specific song choices using only the temporal pattern of the song as a cue, we first allowed them to choose between models of *T. oceanicus* song and the song of a closely related species, *T. commodus* (Fig. 1, D, G, and J). Although the natural songs of these species differ not only in temporal pattern but also in carrier frequency (*T. oceanicus*, 4.5 to 5.4 kHz; *T. commodus*, 3.5 to 4.4 kHz) (*17, 18*), the synthetic models of these songs were presented at identical carrier frequencies (either 4.5 or 5 kHz). The *T. oceanicus* females preferred their conspecific song pattern in both the walking and flight assays (Fig. 2, A, E, and H).

The flight assay appears to be more reliable than the more traditional walking assay. In the walking assay, 35 percent of the crickets chose the "wrong" song, but none did so in the flight assay (*19*).

When *T. oceanicus* females were presented with a choice between their normal conspecific song pattern and shuffled song, they did not discriminate between the two. In the walking assay, nearly equal numbers of crickets walked to each song (Fig. 2B); in the flight assay, most crickets failed to display any discriminatory behavior; that is, they continued to fly straight, as if they received

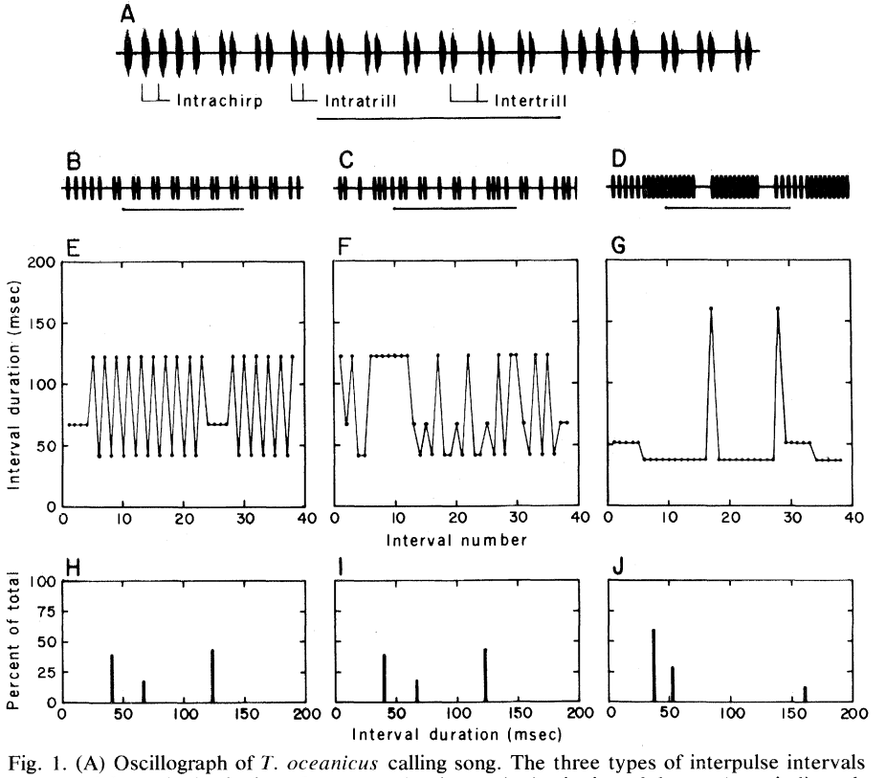

Fig. 1. (A) Oscillograph of *T. oceanicus* calling song. The three types of interpulse intervals (measured from the beginning of one sound pulse to the beginning of the next) are indicated. The horizontal bar represents 1 second. (B-D) Oscillographs of electronically synthesized models of *T. oceanicus* song, shuffled song, and *T. commodus* song, respectively; time bars represent 1 second. (E-G) Sequential interval plots for these three songs. The abscissa represents the ordinal positions of intervals in the song, and the ordinate the durations of these intervals. The regular pattern of intervals present in *T. oceanicus* song is absent in shuffled song. (H-J) The relative proportions of the different intervals in *T. oceanicus*, shuffled, and *T. commodus* songs, respectively.

balanced auditory input from the left and right (Fig. 2F). In a minority of trials crickets did choose one song or the other, but, as in the walking assay, the two songs were chosen equally often (Fig. 2I).

Since *T. oceanicus* females preferred a model of *T. oceanicus* song to a model of *T. commodus* song, if shuffled song were truly interpreted as identical to normal conspecific song then it too should be preferred over *T. commodus* song. In the walking assay, *T. oceanicus* females failed to discriminate between shuffled song and *T. commodus* song (Fig. 2C), but shuffled song was preferred to *T. commodus* song in the flight assay (Fig. 2, G and J).

The failure to distinguish between shuffled song and *T. commodus* song in the walking assay indicates that shuffled song is not interpreted as identical in all respects to normal conspecific song. The failure to distinguish between normal and shuffled song in both the walking and flight assays, however, as well as the preference for shuffled song over *T. commodus* song in the flight assay, argues strongly that *T. oceanicus* females can interpret as conspecific a song (shuffled song) which cannot be matched to an internal copy of the normal song pattern (*20*). Thus, some other mechanism for song recognition must exist either instead of or in addition to such a template-matching mechanism. We stress, however, that our findings do not rule out the possibility that the central nervous mechanisms for song production and recognition might have some elements in common.

We cannot explain why shuffled song was preferred over *T. commodus* song in the flight assay but not in the walking assay. It is possible that, for unknown reasons, the flight assay is more sensitive to small differences in song attractiveness than the walking assay. Such an increased sensitivity would be consistent with the observed absence of errors in the *T. oceanicus*-*T. commodus* discrimination in the flight assay. (Those crickets that walked toward *T. commodus* song in the walking assay might have done so because they chose directions randomly. If the flight assay were more sensitive there would be fewer random choices and hence fewer errors.) Increased sensitivity might permit the detection of a difference in the attractiveness of *T. commodus* song and shuffled song that is too small to be detected in the walking assay.

In addition to its increased reliability (and, perhaps, sensitivity), the flight assay has other advantages. First, statistically significant behavioral expression of a song preference is detectable within minutes, instead of in hours as with the walking assay (*21*). Consequently, data can be collected quickly, and several repetitions of an experiment can easily be done on a single animal. Second, and perhaps more important, the flight assay provides a simple, easily monitored motor correlate of phonotactic behavior.

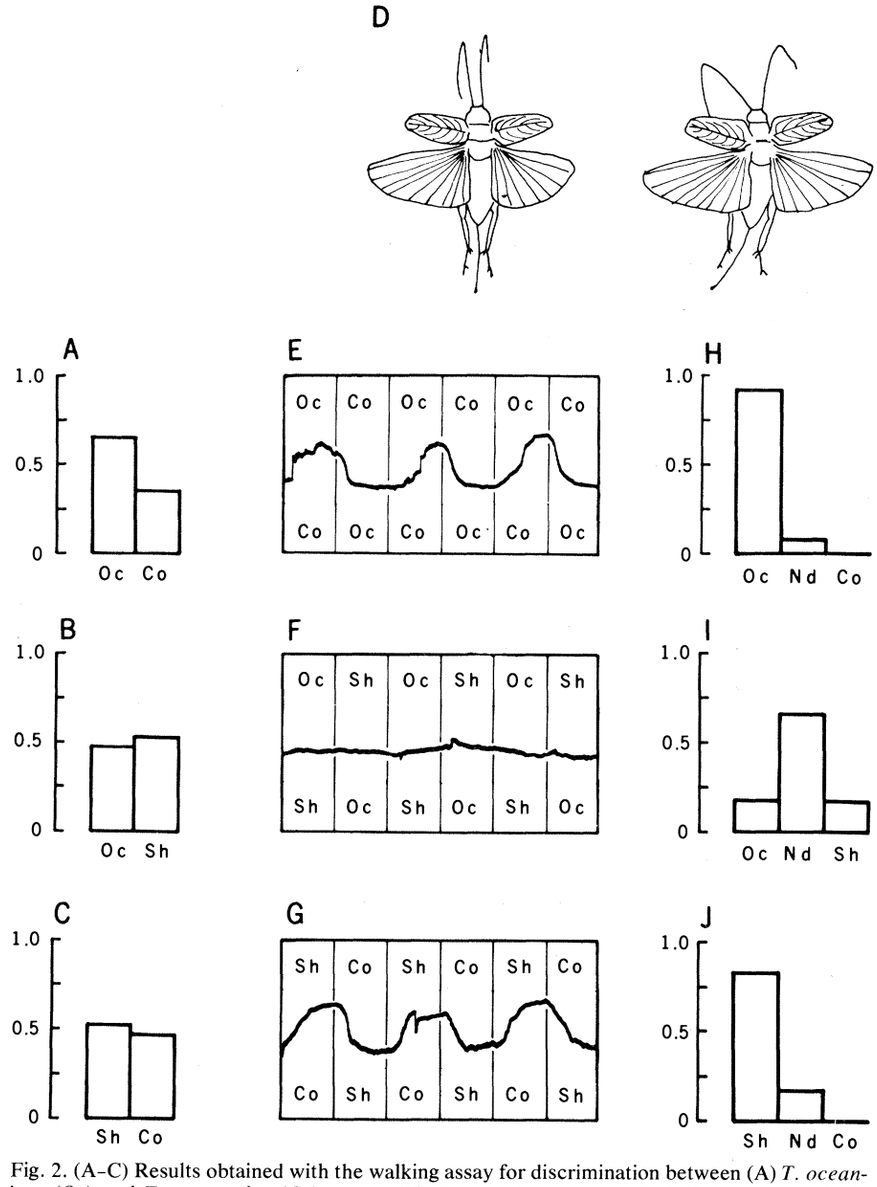

Fig. 2. (A–C) Results obtained with the walking assay for discrimination between (A) *T. oceanicus* (*Oc*) and *T. commodus* (*Co*) songs, (B) *T. oceanicus* and shuffled (*Sh*) songs, and (C) shuffled and *T. commodus* songs. Significant discrimination occurred between *T. oceanicus* and *T. commodus* songs [$P < .05$, *G* test (*24*)] but not between the other song pairs. In (A), $N = 37$; in (B), 148; and in (C), 51; the ordinates represent proportions of responses. (D) Tracings of photographs of a tethered flying cricket (dorsal view), the one on the left with no sound stimulation and the one on the right with synthesized *T. oceanicus* song played from the left. The flexion of the abdomen and hind legs to the left indicate that the cricket is attempting to turn toward the sound source. (E–G) Records of steering movements for a single cricket in the flight assay. A photocell viewing an image of the cricket was arranged so that bending of the abdomen and hind legs to the left resulted in an upward deflection of the trace, and bending to the right in a downward deflection. The song played from the left is indicated above each trace, and that from the right below. The positions of the two songs were reversed at 30-second intervals; the switch points are indicated by vertical lines. The cricket "followed" *T. oceanicus* song in the *T. oceanicus*-*T. commodus* pair (E), failed to follow either song in the *T. oceanicus*-shuffled pair (F), and followed shuffled song in the shuffled-*T. commodus* pair (G) (*25*). (H–J) Results of 24 trials with 12 crickets for each of the three song pairs. The ordinate indicates the proportion of trials on which either one song or the other was followed, or on which no apparent discrimination (no following, *Nd*) occurred. In (H), the most common behavior was to follow *T. oceanicus* song; in (I), failure to discriminate was most common; and in (J), following shuffled song was most common ($P < .005$ in all cases, *G* test) (*26*).

The steering movements monitored in our assay have been studied, in the locust, at the level of single identified motor neurons (*22*). Further, flight behavior survives considerable surgical assault (*23*). It should be possible to record from a cricket's nervous system as it performs acoustic discriminations and thus to correlate neural activity with its behavioral consequences.

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References and Notes

1. T. A. Sebeok, Ed., *How Animals Communicate* (Indiana Univ. Press, Bloomington, 1977).
2. W. H. Thorpe, *Ibis* **100**, 535 (1958); P. Marler and M. Tamura, *Science* **146**, 1483 (1964); P. Marler and S. Peters, *ibid.* **198**, 519 (1977).
3. M. Konishi, *Z. Tierpsychol.* **22**, 770 (1965); ——— and F. Nottebohm, in *Bird Vocalizations*, R. A. Hinde, Ed. (Cambridge Univ. Press, Cambridge, 1969).
4. R. R. Hoy and R. C. Paul, *Science* **180**, 82 (1973).
5. R. R. Hoy, *Am. Zool.* **14**, 1067 (1974); ———, J. Hahn, R. C. Paul, *Science* **195**, 82 (1977); R. R. Hoy, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 2316 (1978).
6. R. D. Alexander, in *Animal Sounds and Communication*, W. E. Lanyon and W. N. Tavolga, Eds. (American Institute of Biological Sciences, Arlington, Va., 1960).
7. D. R. Bentley, *Z. Vergl. Physiol.* **62**, 267 (1969).
8. T. J. Walker, *Ann. Entomol. Soc. Am.* **50**, 626 (1957).
9. M. D. Zaretsky, *J. Comp. Physiol.* **79**, 153 (1972); A. V. Popov and V. F. Shuvalov, *ibid.* **119**, 111 (1977).
10. R. D. Alexander, *Evolution* **16**, 443 (1962).
11. D. R. Bentley and R. R. Hoy, *Anim. Behav.* **20**, 478 (1972).
12. The sequence of intervals was reshuffled every phrase. We produced a 5-minute-long series of unique phrases and repeated this series to make a longer series of phrases for use in experiments. Although trials could last as long as 6 minutes in the walking phonotaxis assay, they rarely exceeded 5 minutes.
13. The trigger pulses activated an electronic switch, which gated the output of a Hewlett-Packard 200-CD wide range oscillator. The resulting pulses had rise and fall times of 5 msec and durations of 30 msec. These pulses were amplified by a stereo amplifier (either Realistic SA-101 or Crown D150A) and played through piezoelectric speakers.
14. The two speakers were 60° apart as viewed from the starting point and were each 122 cm from the starting point.
15. A. Moiseff, G. S. Pollack, R. R. Hoy, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4052 (1978).
16. Sound levels (expressed as decibels relative to 20 μPa) were measured with a 1/4-inch microphone (Bruel & Kjaer 4135) and a sound level meter (Bruel & Kjaer 2209).
17. K. G. Hill, J. J. Loftus-Hills, D. F. Gartside, *Aust. J. Zool.* **20**, 153 (1972).
18. Y. Leroy, *C. R. Acad. Sci.* **259**, 892 (1964).
19. Hill *et al.* (*17*) found that *T. oceanicus* females made nearly no errors in discriminating between natural *T. oceanicus* and *T. commodus* calling songs. The poorer discrimination we observed is due in large part to the absence of frequency difference as a cue. Differences in the geometry or acoustics between our arena and that used by Hill *et al.* may also have contributed to the poorer discrimination we observed.
20. Results similar to ours have been reported for birds. S. T. Emlen [*Behaviour* **51**, 130 (1972)] found that a model indigo bunting song which was temporally rearranged in a manner similar to our shuffled song was as potent as normal song in eliciting reactions from other indigo buntings.
21. Although each trial in the walking assay takes at most 6 minutes, at least five trials are needed to achieve a statistically significant result ($0.5^5 = 0.03$) and far more may be needed if the discrimination is less than perfect.
22. J. J. Dugard, *J. Insect Physiol.* **13**, 1055 (1967); J. M. Camhi and M. Hinkle, *Science* **175**, 550 (1972).
23. D. M. Wilson, *J. Exp. Biol.* **38**, 471 (1961).
24. R. R. Sokol and F. J. Rohlf, *Biometry* (Freeman, San Francisco, 1969).
25. When we judged a trial as exhibiting "following," the behavior was as obvious as that illustrated in (E) and (G): clear changes in abdominal position accompanied each reversal of song positions. When we judged a trial as not exhibiting "following," changes in abdominal position were either absent or small and were not time-locked to reversals in song location.
26. Each of the 12 crickets was tested twice with each of the three song pairs. In nine cases, the behavior of a cricket on the second test with a song pair differed from its behavior on the first test. On all but one of these occasions, one of the trials was judged as "Nd." The single exception occurred on a *T. oceanicus*-shuffled song pair; *T. oceanicus* song was preferred on one test and shuffled song on the other.
27. We thank A. Moiseff for his help with the preparation of the shuffled song trigger pulses and F. Nottebohm, C. Boake, and R. Capranica for their helpful comments on the manuscript. Supported by NIH grant NS-11630-04 to R.R.H. and NIH postdoctoral fellowship IF32NS05541-01 to G.S.P.

29 September 1978; revised 18 January 1979

Voltage Dependence of Junctional Conductance in Early Amphibian Embryos

Abstract. *Isolated pairs of blastomeres from early amphibian embryos* (Ambystoma, Rana, Xenopus) *are electrotonically coupled. Junctional conductance and permeability to the dye Lucifer Yellow are markedly and reversibly decreased by moderate transjunctional polarization in either direction. The relationship between junctional conductance and transjunctional voltage is sufficiently steep that a physiological role in regulation of intercellular communication is plausible.*

It is generally accepted that gap junctions mediate electrotonic coupling and exchange of ions and small molecules between cells. The degree to which ionic current spreads from cell to cell is readily measured electrophysiologically, and in favorable geometries junctional conductance can be unambiguously determined. Conductance and permeability are dynamic properties of the junctional membrane and can be altered by a variety of experimental treatments (*1*, *2*). Substances that can permeate gap junctions conceivably serve regulatory or signaling functions, and control of intercellular flow of small molecules may play an important role in tissue differentiation (*3*). We report here that conductance of junctions between blastomeres of the amphibian embryo is markedly reduced by application of small voltages across the junctions. The sensitivity is sufficiently great that a physiological role in controlling intercellular communication is plausible.

Pairs of blastomeres were mechanically isolated from axolotl (*Ambystoma mexicanum*) or anuran (*Xenopus laevis* and *Rana pipiens*) embryos between the 32-cell stage and late morula. All stages and species showed similar electrical properties. Cells were placed in physiological saline solution (*4*) containing up to 0.05 percent colchicine to inhibit mitosis. Each cell was impaled by two electrodes for applying current and recording voltage.

Intact cell pairs were always electrotonically coupled. When small rectangular current pulses of either sign were applied in one cell, constant voltages were recorded in both cells once the membrane capacity had been charged (Fig. 1, A and B). Larger current pulses resulted in increased input resistance of the directly polarized cell and decreased electrotonic spread to the other cell (Fig. 1, A_2 to A_4 and B_2 to B_4). Essentially identical results were obtained when current was applied in either cell (not illustrated). The coupling coefficients (*5*) could decay from 0.8 or more to 0.1 or less. Uncoupling developed more rapidly with larger polarizations. The cells recovered to their initial state within 1 second after a pulse was terminated. These findings suggest that junctional resistance increases as a function of transjunctional voltage. The nonjunctional membrane of single blastomeres is electrically linear over a comparable voltage range.

In order to measure junctional currents directly, a double voltage clamp procedure was devised. Each cell of a coupled pair was placed in a separate voltage clamp circuit and held at its resting potential (−40 to −60 mV). Voltage steps were then delivered to one of the cells. In this procedure any current flowing via the junctions from the pulsed cell into the second cell is exactly matched by current of the opposite polarity injected into the second cell, which is supplied by its voltage clamp to keep its membrane potential constant. This transjunctional current (I_j) injected into the second cell gives a direct measure of junctional conductance (g_j) when divided by the magnitude of the step change in transjunctional voltage.

Consistent with data from current

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