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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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.
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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).
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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.
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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.

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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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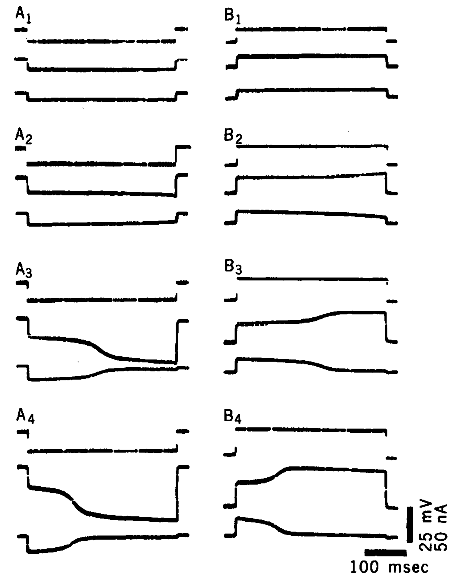
clamp experiments, voltage clamp data show that junctional conductance decreases as a function of transjunctional voltage (Fig. 2). Typically, junctional conductance (1 to 4 μmho) drops to less than 10 percent of its resting level with a 20-mV step (Fig. 2C), but a residual voltage-insensitive conductance persists even at much greater polarizations. Voltage steps of either polarity to either cell produce symmetrical conductance changes (6). It was shown by stepping both cells to different potentials that junctional conductance is independent of potential between the inside and the outside of the cells over a range of at least ± 30 mV from the resting potential. Larger transjunctional voltages cause more rapid decay of junctional conductance (Fig. 2, A and B) (7). The decreased conductance remains stable over pulses as long as 50 seconds.

We have considered that the conductance decrease could be due to accumulation of ions such as H^+ or Ca^{2+} adjacent to the junctional membranes. A simple current-dependent or accumulation mechanism could not account for the phenomenon described here because in the high-conductance condition steady-state transjunctional currents can be passed that are larger than those required to maintain the low-conductance condition (Fig. 2, A_2 and B_2).

If small molecules pass between cells via junctions whose conductance is voltage-dependent, permeability to these molecules should be restricted by voltage differences that cause electrical uncoupling. Pairs of blastomeres were impaled as in the current clamp experiments and Lucifer Yellow (molecular weight, 443) (8, 9) was injected iontophoretically into one cell by hyperpolarizing pulses. When pulses of short duration and small amplitude that did not uncouple were used, dye passage from cell to cell was observed in eight of eight pairs within 10 minutes (10). When Lucifer was injected by longer lasting pulses of larger amplitude at a frequency that held the cells uncoupled, little dye passed from cell to cell over periods as long as 20 minutes (Fig. 3A). These experiments indicate that permeability of these gap junctions to small molecules is voltage-dependent. The results do not permit one to distinguish between a mechanism of conductance decrease in which each member of a large population of conductance elements gradually decreases in permeability, and one in which there is a decrease in the number of conductance elements that are open, each with constant unitary conductance. We cannot yet infer Lucifer imperme-



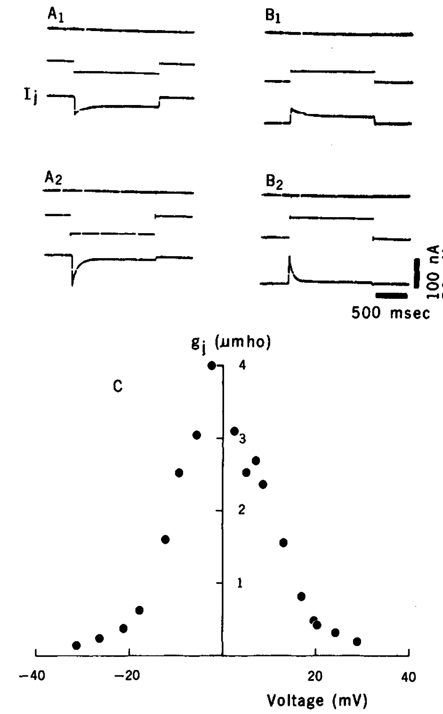
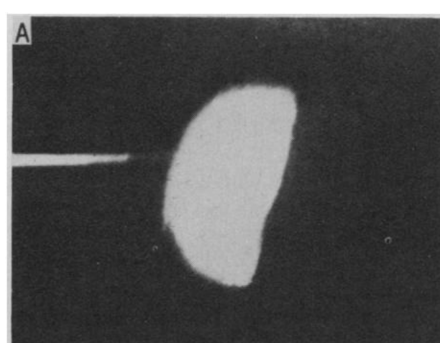
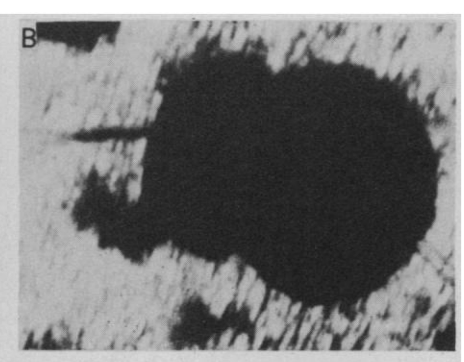


Fig. 1 (left). Electrotonic coupling between a pair of axolotl blastomeres. The upper trace represents current injected into the cell whose voltage is shown on the middle trace; depolarization is upward. Electronic spread to the other cell is shown on the lower trace. For small current pulses (of either sign) the coupling rapidly reached a value that was constant over the duration of the stimulus (A_1 and B_1). For slightly larger pulses the potential in the injected cell began to increase near the end of the pulse, while the potential in the coupled cell decreased (A_2 and B_2). For larger pulses the potential in the polarized cell increased sigmoidally, while the potential in the other cell dropped precipitously (A_3 and B_3). The uncoupling occurred more rapidly with larger pulses (A_4 and B_4). Fig. 2 (right). Voltage dependence of junctional conductance. Each cell of a coupled pair was voltage clamped at its resting potential and voltage steps were delivered to one cell (middle trace, positive upward). Junctional current (I_j) is the current delivered to the other cell to maintain its potential (upper trace, positive current flow into the stepped cell is indicated by a downward deflection in the lower trace). Junctional current fell exponentially to a steady-state value in response to moderate hyperpolarizing (A_1) and depolarizing (B_1) steps. The current fell more rapidly and to a lower level for larger steps (A_2 and B_2). (C) Relation of steady-state conductance to transjunctional voltage. The conductance decreased to about 5 percent of its maximum value with a transjunctional voltage difference of 30 mV.





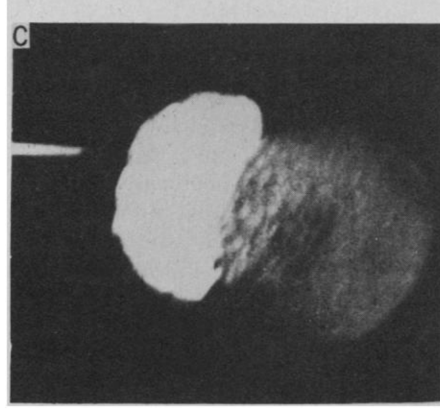


Fig. 3. Voltage dependence of intercellular passage of the dye Lucifer Yellow. Hyperpolarizing current pulses applied to the cell on the left simultaneously injected dye and held the cells uncoupled. After 20 minutes the fluorescent dye was confined to the injected cell (A). When hyperpolarizing pulses were applied to both cells simultaneously, transjunctional voltage was reduced and junctional conductance recovered, allowing dye to cross within a few minutes (C). Fluorescence micrographs (A and C) were obtained with epi-illumination with blue exciting light and 18-second exposures. As shown in the transmitted light micrograph (B), the cells are relatively opaque. The larger cell is 120 μm in diameter.

ability of the residual voltage-insensitive conductance. This component is sufficiently small that passage of Lucifer might not have been detected during the experiment even if the junctions were permeable to it.

The voltage sensitivity described here is found in three amphibian families of two orders. Less marked sensitivity is observed in blastomeres of the teleost *Fundulus* (unpublished observations). Rectification is uncommon at electrotonic synapses, but where it occurs it is generally much faster and probably operates by a different mechanism (*2, 11*). Records similar to those in Fig. 1 have been obtained from pairs of *Limulus* retinula cells, but the morphological basis is unclear and the mechanism may be quite distinct (*2, 12*).

The significance of voltage dependence of junctional conductance remains to be established. One of the major questions about early development is how coupled blastomeres acquire and maintain individual developmental programs. In several instances specific cells or cell groups are known to uncouple or lose their gap junctions at specific times (*13*), and a large difference in resting potential can develop between different regions of an embryo (*14*).

The phenomena described here would allow a cell to determine the extent to which its cytoplasm communicates with that of its neighbors by making small changes in its membrane potential. Changes in relative ion permeability, ion concentration, or electrogenic pumping could all lead to differences in resting potential that rapidly uncoupled the cells from each other (*15*). Additional mechanisms are required to account for disappearance of gap junctions, but the relatively rapid changes reported here provide a possible mechanism for short-term regulation of cellular communication during development.

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4. Physiological saline contained, in millimoles per liter: NaCl, 58.2; KCl, 0.7; $Ca(NO_3)_2 \cdot 4H_2O$, 0.3; $MgSO_4 \cdot 7H_2O$, 0.8; Na_2HPO_4, 0.8; KH_2PO_4, 0.1; and $NaHCO_3$, 0.4 [M. C. Niu and V. C. Twitty, *Proc. Natl. Acad. Sci. U.S.A.* **39**, 985 (1953)].
5. The coupling coefficient is V_2/V_1, where V_1 is the voltage in the cell in which current is injected and V_2 is the potential in the other cell [M. V. L. Bennett, *Ann. N.Y. Acad. Sci.* **137**, 509 (1966)].
6. The voltage-sensitive component of junctional conductance can be well fit by an expression of the form $g_j/(g_m - g_j) = \exp[-A(V - V_0)]$, where g_m is the maximum conductance, V is the transjunctional voltage, V_0 is the voltage at half-maximal conductance, and A is a constant. This result is consistent with a Boltzmann distribution of open and closed channels where $A(V - V_0)kT$ is the energy difference between the two states. The movement of about six electron charges through the entire transjunctional potential would account for the energy differences and the ratio $g_j/(g_m - g_j)$ changes e-fold for a 4-mV change in V. Similar analyses are given for channels in artificial membranes by G. Ehrenstein, H. Lecar, and E. R. Nossal [*J. Gen. Physiol.* **55**, 119 (1970)] and S. J. Schein, M. Colombini, and A. Finkelstein [*J. Membr. Biol.* **30**, 99 (1976)]. The voltage-insensitive component of junctional conductance may arise from a small population of channels which are not voltage-sensitive or from incomplete closure of channels. Alternatively, there may be a small amount of coupling by way of extracellular space in the large area of apposition between cells.
7. Junctional conductance decays exponentially to its steady-state value. As transjunctional voltage increases, the time constant increases then decreases. The recovery of conductance with both cells at the resting potential is also exponential. We conclude that junctional conductance changes by a first-order process.
8. Lucifer Yellow is a substituted 4-aminonaphthalimide dye with high fluorescent yield and good retention by nonjunctional membranes. It was designed by W. W. Stewart [*Cell* **14**, 741 (1978)] for cell marking and has been shown by him and others to cross electronic junctions [Bennett *et al.* (*9*); S. B. Kater and J. J. Galvin, *J. Cell Biol.* **79**, 20 (1979)]. We are indebted to W. W. Stewart for providing the dye.
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10. Impermeability to fluorescein or Procion Yellow has been asserted for gap junctions in several other early embryonic systems [C. Slack and J. F. Palmer, *Exp. Cell Res.* **55**, 416 (1969); M. V. L. Bennett, M. E. Spira, G. D. Pappas, *Dev. Biol.* **29**, 419 (1972); J. T. Tupper and J. W. Saunders, Jr., *ibid.* **27**, 546 (1972)]. The recent demonstration of Lucifer Yellow CH and fluorescein passage in *Fundulus* (*9*) suggests that reevaluation of previous negative and ambiguous [J. D. Sheridan, *Dev. Biol.* **26**, 627 (1971)] findings would be worthwhile.
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15. The voltage dependence is sufficiently great that a decrease in junctional conductance can occur regeneratively under constant-current conditions, such as might be provided by an electrogenic pump. (Compare Fig. 1.) We have seen stable uncoupling induced by brief pulses when resting potentials of cells differed sufficiently. Although in these cases the resting potential differences were probably due to injury produced by microelectrode penetration, greater differences have been observed in situ at later stages (*14*).
16. Supported in part by NIH grants HD-02428, NS-12627, and NS-07512. D.C.S. is a McKnight Scholar in Neuroscience. We are grateful to S. Leber, M. Flomenbaum, J. Jerdan, and P. Model for *Ambystoma* embryos, I. Brick for *Xenopus*, and G. Morrill and A. Kostellow for *Rana*.

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Comparisons of Frogs, Humans, and Chimpanzees

A few minutes with a basic text on amphibians (*1*) reveal that the frogs *Rana* and *Xenopus* differ in at least the following six basic structural traits: (i) tongue (present in *Rana*, absent in *Xenopus*); (ii) centra of anterior vertebrae (procoelus in *Rana*, opisthocoelus in *Xenopus*); (iii) ribs (absent in *Rana*, present in *Xenopus*); (iv) urostyle (articulated to sacral vertebra by a double condyle in *Rana*, fused to sacral vertebra in *Xenopus*); (v) eyelids (functional in *Rana*, nonmovable in *Xenopus*); and (vi) tadpoles (with horny mouthparts and one ventral spiracle in *Rana*, without horny mouthparts and with two lateral spiracles in *Xenopus*). To the extent that we can compare *Pan* and *Homo* with respect to these traits we would find them identical. Moreover, there are no morphological differences between man and chimpanzee of comparable magnitude to those which distinguish the two anurans. An unbiased assessment of morphological differences between *Rana* and *Xenopus* or *Pan* and *Homo* would show just what the genetic data show: trenchant differences between the two frogs and great similarity between the two primates. The external shape comparisons recently presented by Cherry, Case, and Wilson (*2*) seem wanting. By comparing external shape of selected anguilliform vertebrates such as eels (Osteichthyes), snakes and limbless lizards (Reptilia), and caecilians (Amphibia), it could be demonstrated that all of these show greater resemblance to one another than do humans and chimpanzees.

By selecting appropriate anatomical features among vertebrates, one could show great similarity between taxa wide-

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