Supplement 1: simulation software

(**A**) Screenshot of software for performing the particle tracking simulation (Fig. S1). This is the main screen into which the user inputs all of the key parameters for the simulation. (**B**) Matlab code to generate all of the data in this paper. Under different parameters (entered into the user interface shown in Supplement 1A), this software can generate the same characterizations for any electrophoretic system.

Supplement 2: determination of particle number, time interval, and simulation time parameters

To determine those parameters that are not directly constrained by biological measurements (e.g., time granularity of the simulation and number of repeated experiments), we sought those values that caused the final results of the stochastic model to converge. We optimized parameters' values to reduce standard deviation of the right-left gain as much as possible. The true particle number in this system is ~$10^{11\text{-}12}$ (1 pmol serotonin); however this is not computationally tractable. The variation caused by limited number of particles would be $\sqrt{N}/N = 1/\sqrt{N}$; thus, we started with N=2000, which causes variation to be $1/\sqrt{N} = 1/\sqrt{2000} = 2\% < 5\%$. Then, we performed experiments with particle numbers of 200, 20000, 40000, 80000 and 160000 in order to optimize this value. Time interval (the temporal resolution of the simulation) should be as small as is computationally tractable, in order to minimize variability between runs. We started with a time interval of 10^{-3} second, and explored the results of time interval values of 10^{-1}, 10^{-2} and 10^{-4}.

Figs. S2A-F show how the values of parameters were determined (other parameters were as in Table 1). N=20000, t=10^{-2} and simulation time=20 were ultimately chosen for subsequent simulations.

(**A**) We start with t=0.001, repeating 5 times a simulation using particle number N=200, 2000, 20000, 80000 and 160000. At N=20,000, the standard deviation (STD) of right-left gain was smallest. Thus, we chose N=20,000 particles for further simulations.

(**B**) At N=20,000, repeated 5 times, we tested time intervals of 0.1, 0.01, 0.001 and 0.0001 seconds. Smaller time intervals (t=0.1 and t=0.01 second) led to smaller standard deviations (STD) of right-left gain.

(**C-D**) In order to determine which time interval (t=0.01 or t=0.1 second) was best, we further compared the time series of mean and standard deviation (STD) of right-left gain at these two time intervals (Fig. S2C-D). The STD has smaller fluctuation at t=0.01 second. Thus, we chose t=0.01 for further simulations.

(**E**) At t=0.01, we further checked particle numbers N=2000, 20000, 40000 and 80000. The standard deviation of right-left gain is smallest at N=20000. Since further increases of the number did not decrease the STD, we used N=20000 particles, and time interval = 0.01 second for subsequent simulations.

(**F**) Lastly, to determine the correct sample size for our *in silico* experiments, we checked how many repeats of each experiment were necessary to drive the standard deviation to its smallest possible value. At 20 mV, the STD does not decrease any further with >5 repeats of each experiment. But at 40 mV, the STD did decrease with additional repetitions. Thus, we repeated each experiment 20 times in further simulations.

Supplement 3: validation of the model

First we explored how the magnitude of the L-R voltage difference affects the stationary value of morphogen concentration (the final equilibrium state). We modeled the mean stationary morphogen concentrations in the embryo at about 2 hours (105 minutes) under different voltage differences (Fig. S3A): -10 mV, -20 mV, -30 mV and -40 mV. Values for other parameters are listed in Table 1 and are the same as in (Esser et al., 2006). These voltage values represent the differences in transmembrane potential between the right-most and left-most ventral cells which are generated by the fact that the L ventral cell and the R ventral cell perform different ion exchange with the outside medium (Levin et al., 2002; Adams et al., 2006; Levin, 2006; Aw et

al., 2008). Such a voltage difference can be generated by epithelial breaks and other mechanisms in different systems in which electrophoresis may act (Shi and Borgens, 1995).

We used exponential curves to fit the results of concentration and position ($R^2 = 1$ indicating perfect fit), with concentration $C_s = 0.654e^{529.2x}$ with $R^2 = 0.996$; at -10 mV, $C_s = 0.412e^{1044x}$ with $R^2 = 0.998$ at -20 mV, $C_s = 0.248e^{1563x}$ with $R^2 = 0.999$ at -30 mV, and $C_s = 0.143e^{2080x}$ with $R^2 = 0.999$ at -40 mV. These relations can be also derived by the deterministic model (Esser et al., 2006) as

$$C_s = C_s^0 \frac{z_s FV}{RT} \frac{\exp\left(-\frac{z_s FVx}{RTL}\right)}{1-\exp\left(-\frac{z_s FV}{RT}\right)} \quad (4)$$

According to this equation, the expected relations are $C_s = 0.656e^{528x}$ for -10 mV, $C_s = 0.409e^{1056x}$ for -20 mV, $C_s = 0.243e^{1584x}$ for -30 mV, and $C_s = 0.139e^{2112x}$ for -40 mV. We conclude that the stationary concentrations have an exponential relation with the distance to the left border; thus, the results from the stochastic model are consistent with that of the deterministic model.

To determine how the magnitude of the voltage difference affects the stationary value of morphogen right-left gain R_s (ratio of concentrations of right-most and left-most cells), we calculated, as a function of voltage, the mean and standard deviation of the right-left gains within 2 hours (Fig. S3B). The gains reach their maximum values in about 40 minutes and have bigger standard deviations with higher voltage differences. R_s has a mean value 20.90 at -40 mV (-39 mV in fact), 9.85 at -30 mV (-29.25 mV in fact), 4.65 at -20 mV (-19.5 mV in fact) and 2.17 at -10 mV (-9.75 mV in fact), we fit the data by exponential curves and have: $R_s = 1.023e^{77.4V}$, and $R^2 = 1$; this matches the relationship derived by the deterministic model (Esser et al., 2006) as: $R_s = e^{-z_s FV/RT} = e^{79.23V}$. We conclude that right-left gain has an exponential dependence on the voltage difference, and again, that the stochastic model reproduces the behavior of the deterministic model.

In order to determine how the diffusion constant affects morphogen concentration, we first studied the temporal change of the morphogen concentration with left-right voltage difference of -20 mV and diffusion constant $D_s = 3 \times 10^{-10}\ m^2/s$ (Fig. S4A); these values are estimated from or measured in experiments (Levin et al., 2002). The concentration reaches its stationary value at about 40 minutes and the profile is monotonic. To determine how diffusion affects right-left gain, we studied the mean and standard deviation of the right-left morphogen gains under different diffusion constants (Fig. S4B). The mean values from different diffusion constants converged at about 105 minutes. Morphogen gains in runs using smaller diffusion constants ($D_s/5^{1/2}$, for a molecule with five times the molecular mass of serotonin, if $D \propto 1/\sqrt{M}$ (Weiss, 1996; Esser et al., 2006)) increased more slowly than in those using the larger diffusion constant (D_s). We conclude that slower diffusion constant cause the concentration and gain to require a longer time to reach their stationary state; the time to reach maximum gain is thus diffusion-limited. Since the diffusion constant decreases with increasing mass (assuming larger mass is related with bigger radius) and the viscosity (diffusion coefficient $D = \frac{k_B T}{6\pi\eta r}$ for a sphere with radius r, where η is the viscosity of the medium), a larger morphogen mass and higher viscosity will make the morphogen move slowly. For example, for a morphogen with double the mass of serotonin (its diffusion constant is $D' = D_s/\sqrt[3]{2} = 0.8D_s$), its time to reach the maximum

gain increases by a factor of $\sqrt[5]{2}$, that is 55-60 minutes; the maximum gain reached after 55-60 minutes is the same. This has implications for using fluorescent labels; given the small size of serotonin, our results show that attaching a fluorescein or Alexa^{tm} label (for *in vivo* tracking of 5HT movement) would significantly alter the dynamics of the gradient.

In order to determine how the morphogen gradients change with respect to time, we studied the temporal changes in morphogen gradients in the embryo and in each cell (Fig. S5). The gradient in the whole embryonic field increases rapidly and smoothly during the time course; however, variability is observed inside individual cells (including decreases in gradient, such as observed in $cell_1$). The gradient at each position actually can be calculated by the deterministic model:

$$gradient = \frac{dc_s}{dx} = \frac{c_0^2}{L}\left(\frac{z_i F V}{RT}\right)^2 \frac{\exp\left(-\frac{z_i F V x}{RTL}\right)}{1-\exp\left(-\frac{z_i F V}{RT}\right)} = 431\exp(1056x) \qquad (5)$$

We conclude that, counter-intuitively, simple electrophoresis can produce highly complex spatio-temporal pattern of gradients at two scales. In this, again the stochastic model of individual particle behavior is shown to be consistent with the deterministic model of the overall gradient.

We next added cell boundaries and gap junctions, and studied the spatiotemporal change of morphogen concentration and right-left gain under different numbers of gap junctions (and different gating probabilities). To determine how gap junctions affect morphogen distribution, we first studied the spatio-temporal change of mean morphogen concentration with fixed gap junction number $N_{GJ}= 10^6$, and gate open probability = 1. Mean morphogen concentrations form zigzag slopes at first, and then reach smooth profiles later with a few more particle accumulating at cell-cell boundaries (Fig. S6). This is different from the behavior in a syncytium without cell boundaries (Fig. S4A) and is likely due to the particles' accumulating on the left side of the cell membrane before eventually passing to the other side of the membrane through gap junction channels. When the time is sufficient, the morphogen concentration can still be formed properly. We conclude that the presence of gap junctions, even if performing as simple passive pores, provides an additional level of complexity in the resulting morphogen concentration profile over a syncitial cytoplasmic compartment.

To determine how gap junctions affect the right-left gain of morphogen gradient, we studied the temporal change of right-left gain and/or its standard deviation under different gap junction densities (Fig. S7A-B). The mean morphogen right-left gain reaches its stationary value quickly with more gap junction channels (equivalent to higher open probability or larger channel pore size) (Fig. S7A) and the gain reaches its stationary value at $N_{GJ}=10^6$ in the same time interval (four hours) as at $N_{GJ}=10^7$ (Fig. S7B). We conclude that the particle distribution is effectively controlled by changes in GJC of greater than two orders of magnitude; smaller changes in gating probability (physiological values are 0.2-0.99 (Veenstra et al., 1995; Goldberg et al., 2004)) do not strongly affect the left-right gain.

We next determined how gap junctions affect the temporal change of morphogen gradient at embryonic and cellular levels by plotting the temporal change of morphogen gradient in each cell (Fig. S8). The morphogen gradient in the embryo increases smoothly during the timecourse (from blue to cyan, in the pseudocolor scale); however, at the cellular level, we observed non-monotonic changes in some cells. $Cell_{2\text{-}4}$ and $Cell_6$ especially showed increases/decreases and then decreases/increases of the gradient. Small variability in the temporal profile of the gradient was observed among the different blastomeres, even though they form a symmetric, linear path

between the poles of the electrophoretic field and thus would not be expected to exhibit complex (non-monotonic) differences among the blastomeres (Fig. S8B). We conclude that electrophoresis across a linear field can result in complex and different spatio-temporal changes among individual cells, while the overall gradient across the field rises smoothly.

Compared to Fig. S5B (syncitium condition), we found the gradient at the embryonic level to require more time (100 minutes in Fig. S8B, 50 minutes in Fig. S5B) to reach the final magnitude, while the gradient at cellular level is built up very early (Fig. S6). We conclude that cell boundaries (even with gap junctions) significantly delay the appearance of an embryo-wide gradient but the intracellular gradients can appear very quickly in electrophoretic systems.

In order to determine how particle properties and degree of GJC affect stationary/transitional values of morphogen concentration, we studied the spatial concentration profile at about 105 minutes with different diffusion constants (Fig. S9A) and gap junction numbers (Fig. S9B). Diffusion constants were not a significant factor in determining the profile (Fig. S9A). In Fig. S9B, under $N_{GJ} = 10^{2}$, cells have uniformly polarized morphogen distribution (gradient); with $N_{GJ} = 10^{5\text{-}6}$, cells on the left exhibited low morphogen gradients while cells on the right exhibited high morphogen gradients (Fig. S8A). The gradient across the whole embryo cannot form in 2 hours with low GJC. We conclude that although the stationary morphogen profile and gradient are not determined by gap junction number, the number of gap junction channels does affect the transient spatial profile of the morphogen distribution.