Successive Induction in Larval Zebrafish

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ABSTRACT

Activating one reflex often facilitates another, antagonistic one. Since Charles Sherrington first identified *successive induction* more than 100 years ago, it has been demonstrated in a wide range of species, from aphids to grasshoppers to dogs and humans. We show a particularly orderly example in zebrafish (*Danio rerio*) larvae and identify the simple dynamic process that seems to underlie it.

Charles Sherrington, more than one hundred years ago, studied the reflexive behavior of a variety of decerebrate mammals. From his own work and the work of others he identified a set of reflex properties such as latency, habituation, and refractory period, that can be observed in all animal species. Sherrington also identified the properties of interaction between reflexes. One of the most interesting is *successive induction*, which is the facilitation of one reflex by the preceding excitation of an antagonistic one. For example, "…the extension-reflex predisposes to and may actually induce a flexion-reflex, and conversely the flexion-reflex predisposes to and may actually induce an extension-reflex"[i, ii].

Successive induction (also known as *contrast*) has been identified in a variety of species with a range of responses. In response to changes in illumination, for example, aphids (*Aphis fabae*) show 'antagonistic induction' and 'antagonistic inhibition': under appropriate conditions, flight induced by light enhances the subsequent settling response, and settling reciprocally enhances the photokinetic response[iii]. A similar pattern of activity change in response to change in illumination has been shown in locusts (*Locusta migratory migratorioides*,[iv] and most recently in zebrafish larvae (*Danio rerio*)[v], and related research cited therein.

Sherrington's analysis of the properties of single reflexes led him to postulate the existence of the synapse, a junction between nerves with well-defined properties. The synapse was subsequently observed directly, but the process underlying successive induction has not been identified, and its physical basis has not been established. In this note we describe some new data on successive induction and a simple theory for its operation.

MacPhail et al. (2009) measured the movement of individual zebrafish larvae (6 days post-fertilization [dpf]) separately housed in 96-well microtiter plates. The movement (cm/unit time) of each larva as a function of changes in the level of visible vs. infrared light (perceptual dark) was recorded with a video-tracking system. The average data are extremely orderly and highly repeatable. The basic results are:

1. In the dark (infrared illumination only) not preceded by light, activity level is low and constant.
2. In the light, activity level slowly increases to an intermediate asymptote.

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3. In dark, preceded by a period of light, activity reaches a high level and then slowly declines. This is an example of successive induction: suppression of activity in the light facilitates activity in subsequent dark.

These properties are summarized in Figure 1, which shows the effect of continuous dark (red triangles), continuous light (open circles) and alternating light and dark (white/red squares) on the activity level of groups of zebrafish larvae.

The Process

The process can be modeled by a system of two leaky reservoirs. First consider reservoir **B** in Fig. 2 and let the water level in **B,** x_2, represent activity level. In the light, activity slowly increases to an intermediate asymptotic value. This behavior can be modeled by **B** filling at a steady rate with an outflow determined by its level: the higher the level, the faster the outflow, yielding an equilibrium level where inflow is equal to outflow (k_2x_2 in Fig. 2) where x_2 is the level and k_2 is, in effect, the size of the leak. If inflow occurs only during light, we have then a simple model for behavior in the light (open circles in Figure 1).

To capture successive induction, however, the inflow to **B** must be the outflow from a second (upstream) reservoir, **A**, which fills up only during the light, and mainly empties into **B** in the dark. The large activity (x_2) increase observed in the dark after a period of light implies that inflow to **B**, in fact, comes from **A**, which fills at a steady rate (V, say) only during the light period.

If **A** empties only slowly in the light (k_{1L} is small), it will fill up during the light. The increase in activity in dark after a light period then corresponds to an increase in the **A** $\rightarrow$ **B** discharge rate in the dark ($k_{1D} >> k_{1L}$). In other words, **A** fills up in the light, but also leaks (slowly) into **B**, yielding the slow increase in **B** level (i.e., activity) in light. But in dark, the discharge rate from **A** $\rightarrow$ **B** increases, thus producing an increase in the level of **B** (i.e., an increase in activity). The increase in the level of **B** is transient, however, because V (inflow to **A**) is assumed to equal zero in the dark – so that **A** eventually empties, the flow into **B** ceases, and then **B** also empties (activity declines to zero).

These events are combined in Figure 2, which is a physical analog to the process. It has the following ingredients:

1. x_2 corresponds to observed activity level.
2. The inflow into reservoir **A** is at a constant rate V that is positive when the light is on and zero when it is off.
3. **A** leaks into reservoir **B** at a rate proportional to its level, x_1, and the setting of the valve (size of the leak) $k_{1D/L}$, whose value depends on light level, large in dark, small in light: $k_{1D} >> k_{1L}$
4. **B** leaks at a rate proportional to its level, x_2, and the size of the leak k_2. k_2 is constant.

We hope this physical description will adequately explain the relatively simple process that is described more formally by the following two difference equations:

$$\begin{aligned} x_1(t+1) &= V + x_1(t) - k_{1D/L}x_1(t), \\ x_2(t+1) &= k_{1D/L}x_1(t) + x_2(t) - k_2x_2(t), \end{aligned} \qquad (1, 2)$$

where t is discrete time, V is in effect a scale parameter and k_1 takes on different values in the dark and light: $k_{1D} >> k_{1L}$, and $V = 0$ in dark and $V > 0$ in light.

Equation 1 describes the flow into and out of reservoir *B*; x_1 will obviously have a maximum (asymptote) at V/k_{1L}. The outflow from **A**, $k_{1D/L}x_1$, is the inflow to **B**. The level in **B**, similarly, has an asymptote at $x_2/(1-k_{1D/L}+k_2)$. In the light, because k_1 is then small, the effective asymptote is $x_2/(1+k_2)$.

Thus, after a period in the light, x_1 will be large and x_2 will be small. When the light goes out, k_1 increases allowing a large increase in k_1x_1 and thus an increase in x_2. But, because V is zero in the dark, k_1x_1 soon diminishes, and, after a lag, so does activity, x_2.

Tests

We look first at the qualitative fits between model and data and then look at more quantitative fits.

Qualitative fits. *Preceding dark time has no effect*: Figure 3 (left panel) shows data from two experiments in which the larvae were exposed to 20 min of bright light (51.9 lux) preceded by either 10 minutes or 20 minutes of dark, and followed by 10 minutes of dark. The curves are identical, showing (a) the high replicability of the data with this preparation; (b) successive induction: activity in the dark is elevated by prior light, and (c) that duration of the initial dark period has no effect, which is as the model predicts because inflow, *V*, is zero in the dark.

More light yields more activity in subsequent dark: Figure 3 (right panel) shows activity in 20 minutes of dark preceded by either 5 or 15 minutes of light (i.e., x_1 is larger). There is more activity in the dark when preceded by a longer light period, as the model predicts. Figure 4 shows a simulation of this experiment. The pattern is the same, more light yields more subsequent activity, but the peak of the 15-min-light line is roughly 3 times higher than the 5-min peak, rather than about 1.5 times higher, as in the data in Figure 3.

Quantitative fits: Figure 5 shows successive induction in 10 minutes of dark following 20 minutes of light (triangles); the magenta line shows a simulation, for which the parameter values were fitted by inspection. The model readily captures the approximately linear increase in activity in the first few minutes of light as well as the rise-and-fall in subsequent dark.

Figure 6, top panel, shows a quantitative fit to a complicated light-dark series, shown by the on-off graph at the top. Note particularly the sharp response to a single, brief (60-s) light period (at 30 min). Figure 6, bottom panel, shows the reverse effect – of a brief dark period (at 20 min). The parameters for both fits are the same, only the initial conditions are different. Both graphs show that the fit is good for the first 30-40 min, although the model tends to over-predict activity in the latter portions of the experiments.

DISCUSSION AND CONCLUSION

The average activity-level changes caused by light-dark changes in this procedure are highly replicable. Moreover, the process represented by Equations 1 and 2 captures the essentials of the effect of light and dark on activity in zebrafish larvae, at least for the first 30-40 min or so of the experiment. Once the procedure was completely standardized, as it was for the two separate experiments shown in Figure 6, the same simulation parameter values seem to apply for any on-off input sequence.

The model fails, however, in two respects. First, the difference between the relative peaks in Figures 3 (right panel) and 4 shows that the assumption that x_2 is directly related to measured activity level is too simple, Simulated activity level (x_2) increases too much with additional time in light. This discrepancy is independent of particular parameter values; it is a consequence of the linearity of the model. For a comprehensive model it is both plausible

(activity surely does have an upper limit) and necessary to include a non-linear limiter for rate, i.e., some negatively accelerated function relating x_2 to actual activity.

Second, the model over-predicts dark activity in the latter part (> 35 min) of the two experiments shown in Figure 6. This discrepancy also cannot be eliminated by changing parameter values without impairing the fit earlier in the experiments. Because predicted and actual activity levels are high at the end of these experiments, a rate-limiting assumption for x_2 would improve the model fit here also, but at the cost of increased complexity.

Our aim at this stage, however, is not so much to provide a necessarily complex model that fits every detail as to expose the essential process that seems to underlie successive induction in these experiments. That process clearly involves a hidden variable (x_1) that increases during time in the light and then facilitates activity in subsequent dark in a fashion consistent with a depleting store. The relative simplicity of the process, like the simplicity of the synapse identified by Sherrington, suggests a comparably simple underlying physiology. We look forward to new research at the neural and molecular levels that might elucidate further these striking effects.

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FIGURE LEGENDS

1. Successive induction in response to light changes in 6-dpf zebrafish larvae. Average activity level (measured as distance moved in 2-min periods) increases to an intermediate asymptote in continuous light, rises to a high level then declines in the dark following a light period, or stays at a low level in continuous dark not preceded by light (experimental details in MacPhail et al., 2009). Reproduced with permission of the journal publisher.

2. A physical analog to successive induction. Level in reservoir **B**, x_2, corresponds to activity level. Reservoir **A** fills up at a steady rate V which is directly related to light level. **A** drains into reservoir **B** at a rate k_1x_1, which is determined by light level: k_1 is small in the light and large in the dark. Reservoir **B** drains at a constant rate k_2. Thus, in the light, **A** fills up. In subsequent dark, **B** rapidly fills (activity level rises) and then declines.

3. Left panel: Lack of effect of two different prior dark periods on subsequent light and dark. Right panel: Effect of light-period duration (longer light yields more activity in subsequent dark). Horizontal bars indicate periods of light (unfilled) and dark (filled). From MacPhail et al. (2009). Reproduced with permission of the journal publisher.

4. Effect of light-period duration (simulation of results in right panel of Figure 3). Parameters: $V = 5$, $k_{1D} = 0.2$, $k_{1L} = 0.04$, $k_2 = 0.9$; initial conditions: $x_1(0)$, $x_2(0)$, both set equal to 0. Peak ratio is proportional to prior light-duration ratio. Horizontal bars indicate periods of light (unfilled) and dark (filled).

5. Data (triangles): Effect of 20-min light on activity in subsequent dark. Activity increases approximately linearly in light then increases rapidly and declines in the dark. Simulation (line) used the following parameter values: $V = 5$, $k_{1D} = 0.2$, $k_{1L} = 0.04$, $k_2 = 0.9$, initial conditions: $x_1(0)$, $x_2(0)$, both set equal to 0. Horizontal bar indicates periods of light (unfilled) and dark (filled).

6. Top panel: Effect of repeated light and dark periods on activity level (blue triangles) and simulation (line) – note the effect of a brief light period at 30 min and a longer light period beginning after 60 min. Bottom panel: Effect of repeated light and dark – note the effect of a brief dark period at 20 min. Horizontal bars in both panels indicate periods of light (unfilled) and dark (filled). Parameters for both simulations (blue line) were: $V = 5$, $k_{1D} = .07$, $k_{1L} = .01$, $k_2 = .8$. Initial conditions were $x_1(0) = 15$ for the top graph and 20 for the bottom (to account for the initial burst due to unmeasured effects); $x_2(0) = 0$ for both.

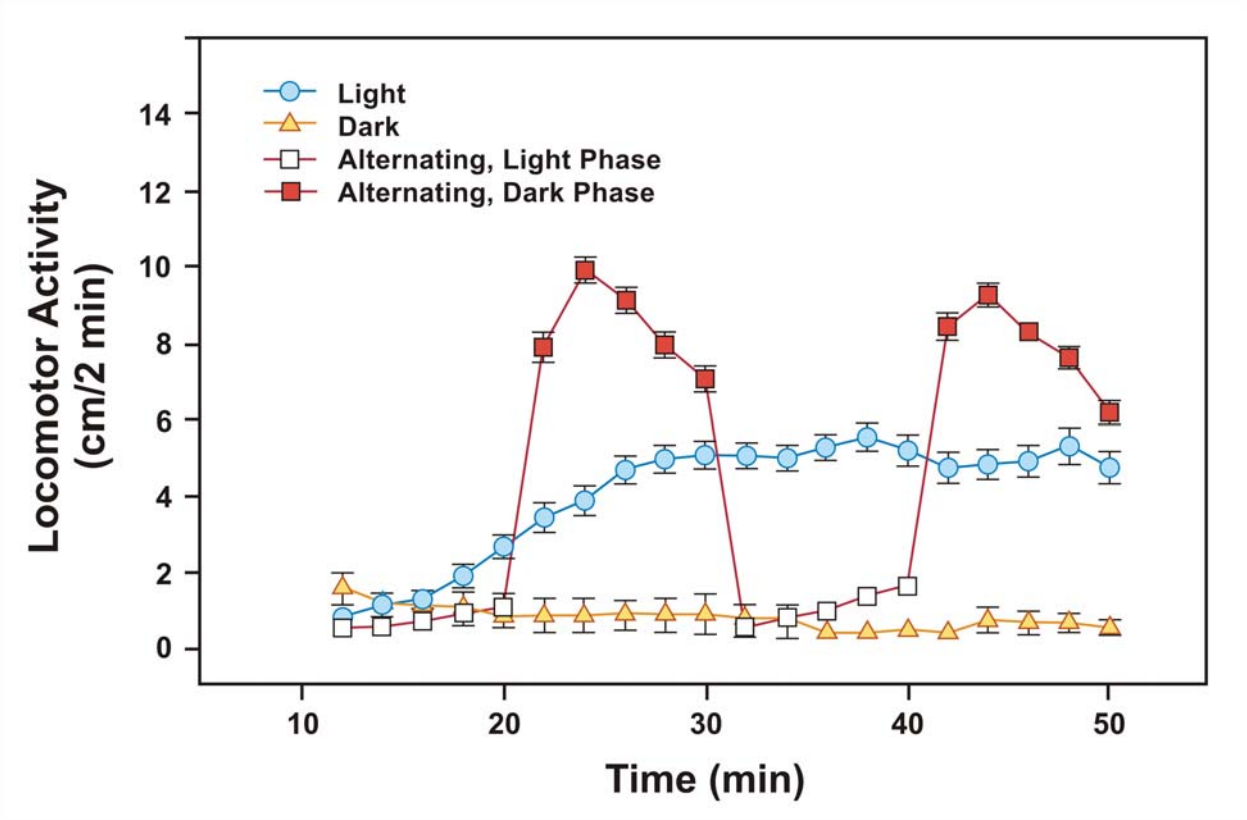


Figure 1

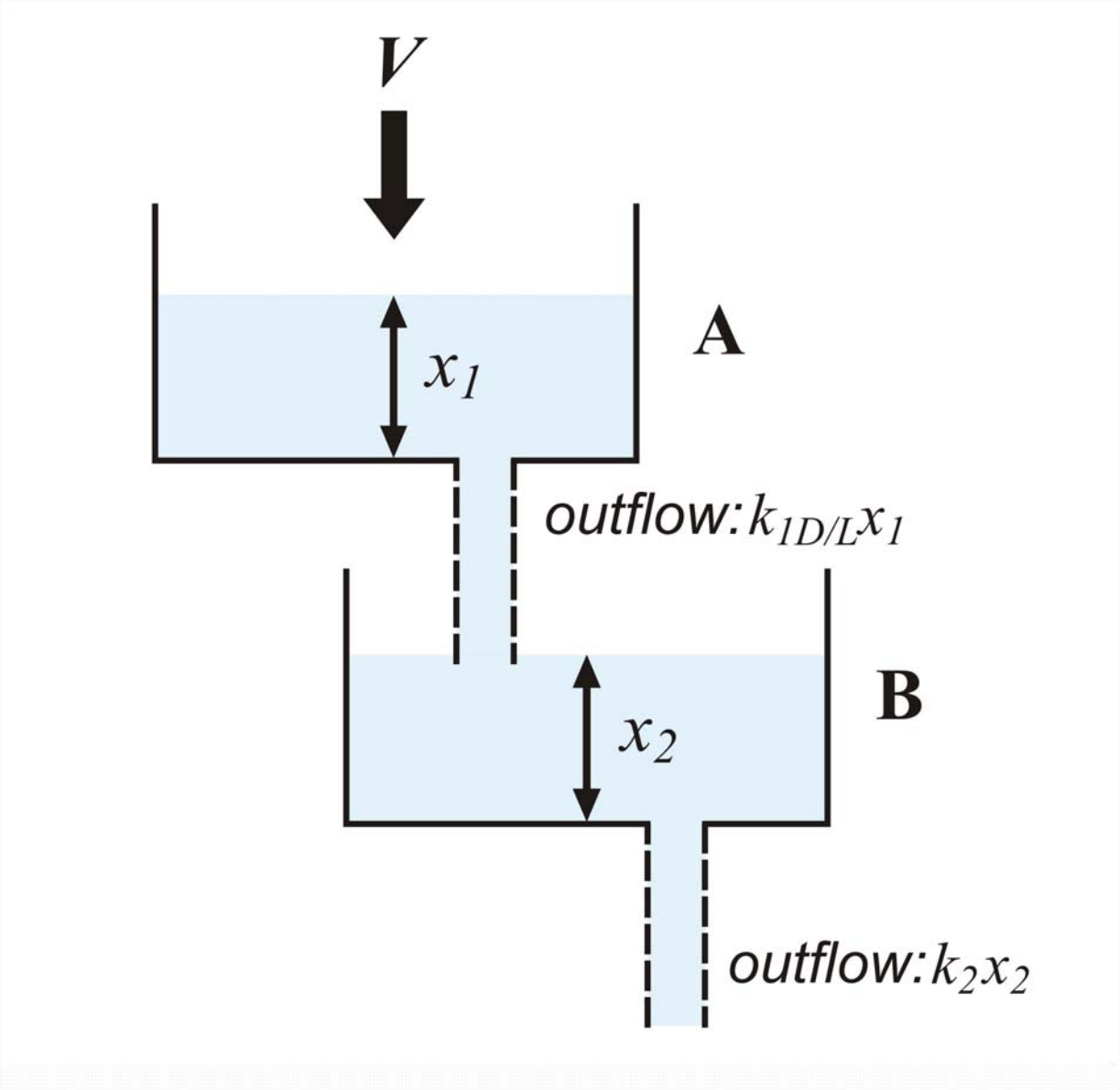


Figure 2

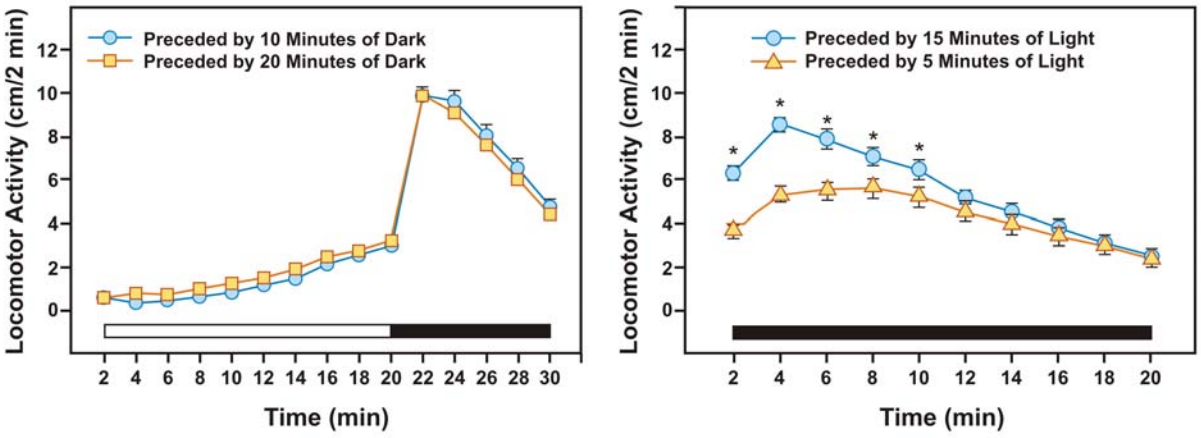


Figure 3

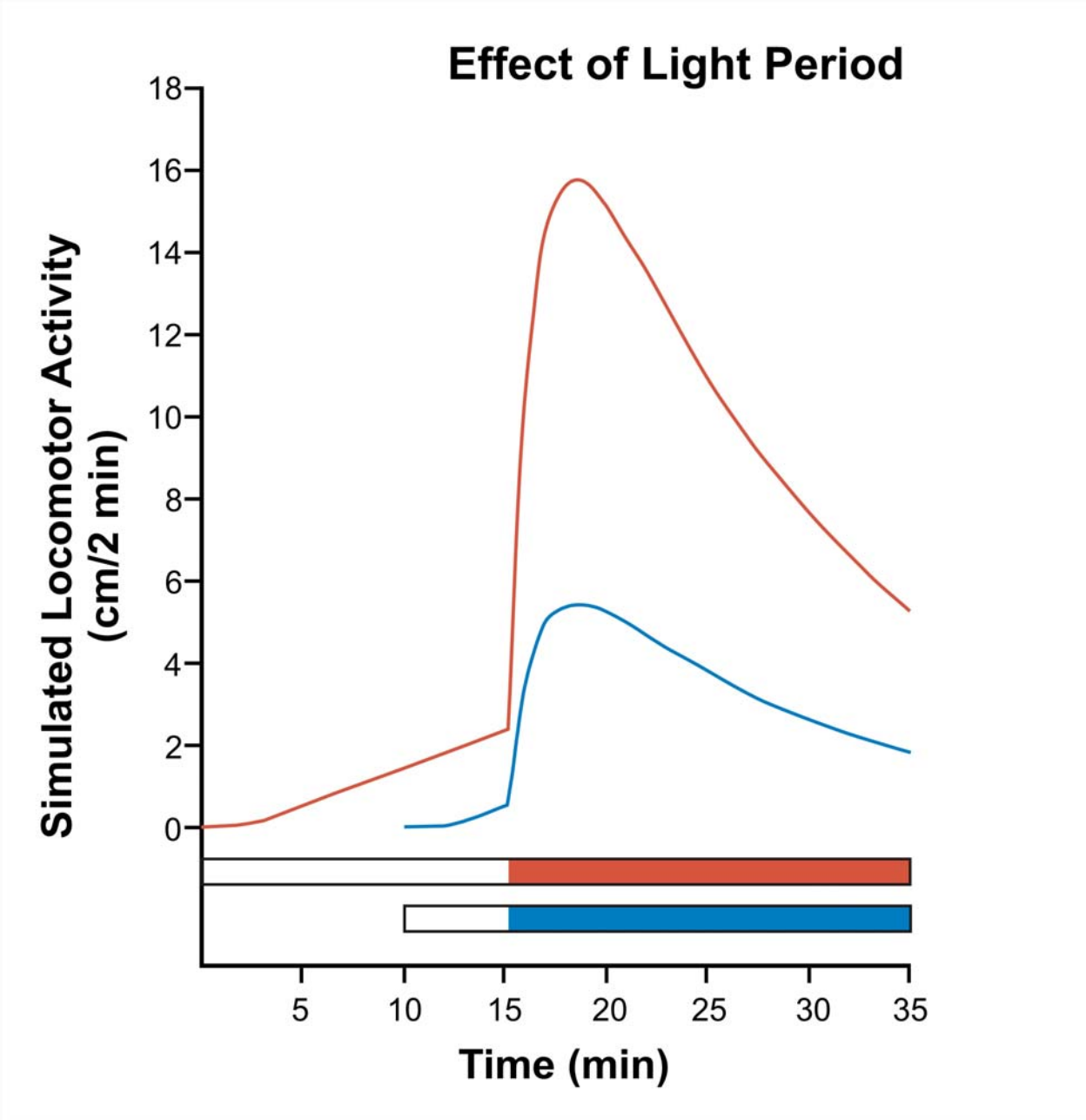


Figure 4

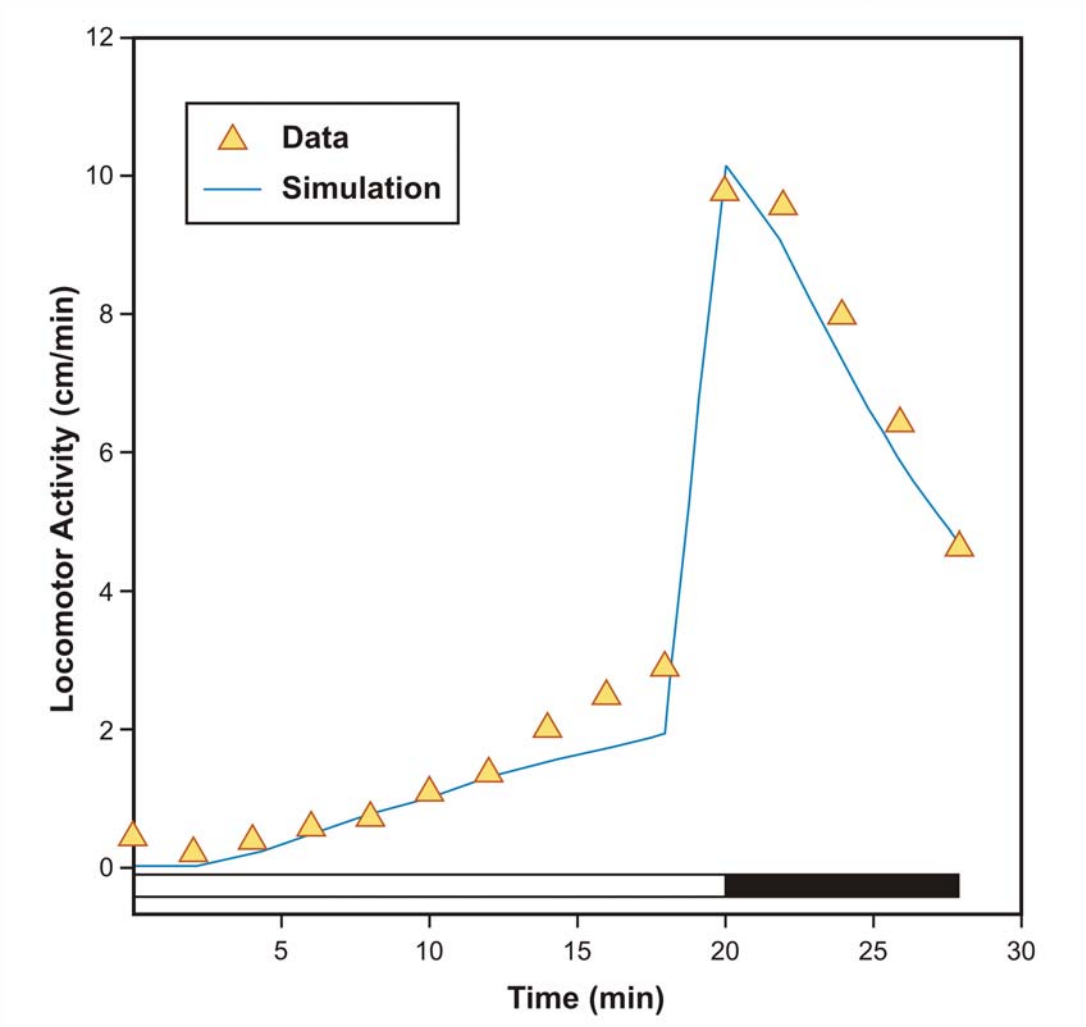
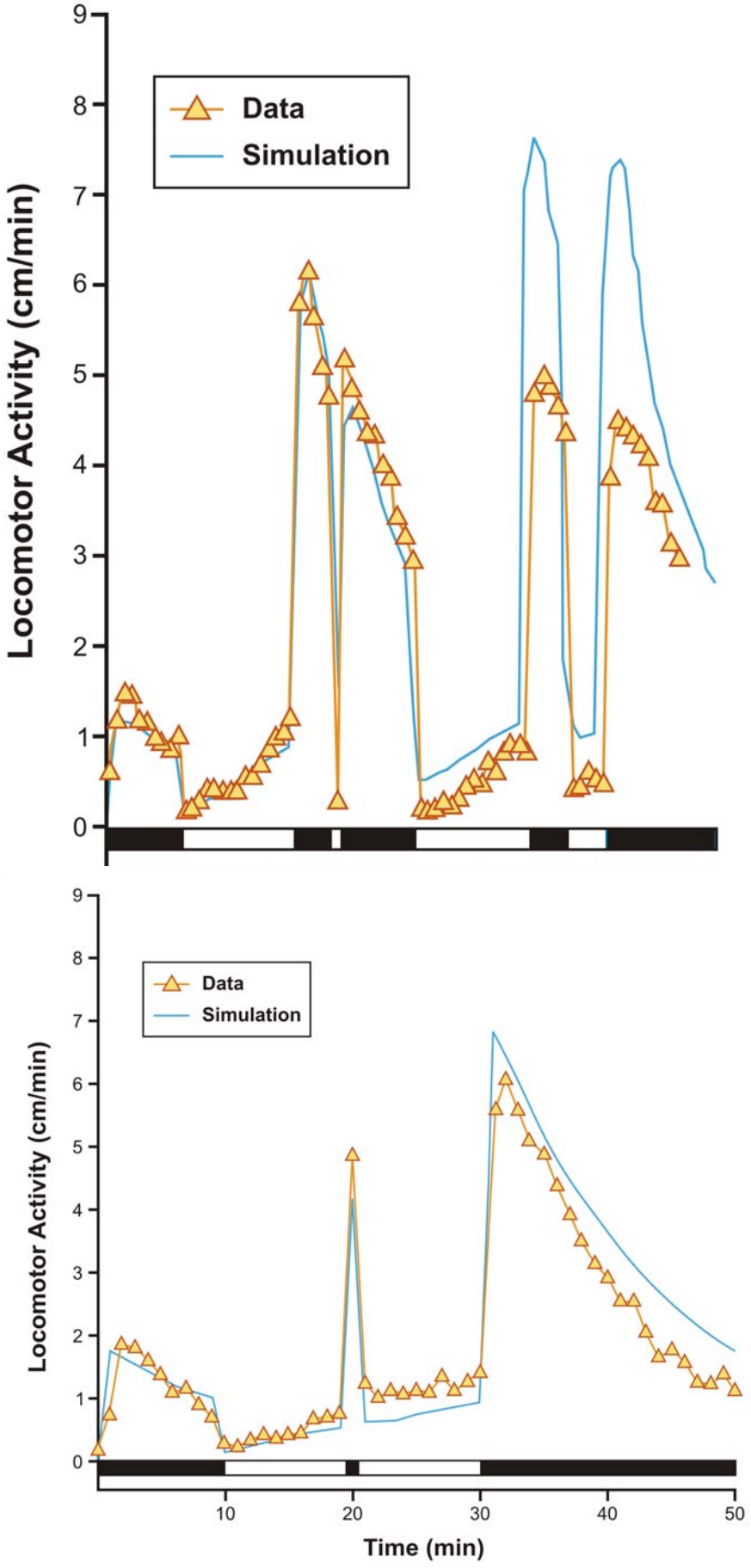


Figure 5

Figure 6



FOOTNOTES

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