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A Simplified Model of the *Drosophila melanogaster* Circadian Oscillator: A Closer Look at the Role of Feedback Loops and Time Delays

Abstract

In this study, we model the core transcriptional-translational feedback loop (TTFL) underlying circadian oscillations in *Drosophila melanogaster*. Specifically, we focus on the interaction between the transcription factor *dCLOCK* and its target protein *PER*. Our main objective is to understand how negative feedback, mediated by *PER*, generates and sustains rhythmic protein expression. To do so, we developed a minimal mechanistic model based on ordinary differential equations (ODEs), where *dCLOCK* promotes *PER* synthesis, and accumulated *PER* inhibits its own expression by sequestering *dCLOCK*. The model was simulated using numerical ODE solvers to explore system dynamics under varying parameters and feedback strengths. We further extended the model to include stochastic differential equations (SDEs) to capture biologically realistic fluctuations arising from molecular noise. Our key findings show that delayed negative feedback is both necessary and sufficient to generate sustained oscillations, while the inclusion of positive feedback improves the precision and robustness of the rhythm. The SDE simulations reveal that the system maintains oscillatory behavior even under moderate stochastic perturbations, highlighting the intrinsic robustness of circadian regulation. These results demonstrate that even minimal models can capture essential circadian dynamics, suggesting an evolutionary advantage in using simple, efficient regulatory motifs. Biologically, our study sheds light on the fundamental mechanisms driving circadian rhythms and how their disruption could lead to health and behavioral disorders.

Introduction

Circadian rhythms are 24-hour biological oscillations that regulate essential physiological and behavioral processes, including sleep-wake cycles, feeding, metabolism, and hormone secretion. These rhythms are found across nearly all living organisms, from cyanobacteria to humans, and are driven by molecular feedback loops that generate self-sustained oscillations. In *Drosophila melanogaster*, the core molecular oscillator consists of interlocked transcriptional-translational feedback loops (TTFLs), with key components including the transcription factor *dCLOCK* and the protein *PER (Period)*. *dCLOCK* activates the transcription of *PER*, while *PER* protein accumulates in the cytoplasm, undergoes post-translational modifications, and translocates into the nucleus. This represses *PER's* own transcription by inhibiting the *dCLOCK/CYCLE* complex. These oscillations in circadian rhythms are remarkably robust despite being driven by inherently noisy processes. Disruptions in circadian timings are linked to a wide range of disorders, including sleep disturbances, metabolic syndrome, and neurodegeneration. Studying *Drosophila*'s circadian clock provides insight into conserved mechanisms that govern biological timekeeping in more complex organisms, including humans.

Our goal is to model the *PER/dCLOCK* feedback loop using a system of ordinary differential equations (ODEs) that captures the essential dynamics of transcription, translation, and nuclear feedback inhibition. Specifically, we aim to (1) determine how delayed negative feedback sustains oscillations, (2) examine how positive feedback influences rhythm robustness, and (3) analyze how perturbations and molecular noise affect system stability. To accomplish this, we also extend our ODE model using SDEs, allowing us to incorporate intrinsic biochemical noise and assess its influence.

Previous modeling efforts have used both delay differential equations (DDEs) and stochastic models to capture circadian dynamics. Leloup and Goldbeter (1998) introduced DDE-based models to explicitly incorporate transcriptional and translational delays, successfully reproducing oscillatory behavior. However, DDEs can be analytically complex and difficult to connect to specific biological mechanisms. More recent work has shifted toward ODE-based models with intermediate steps that approximate biological delays while improving tractability and interpretability (Mirsky et al., 2009). Additionally, stochastic modeling approaches (Forger & Peskin, 2005) have shown that noise can significantly impact oscillatory dynamics, but few studies have integrated noise into simplified, biologically interpretable core-clock models.

In this study, we constructed a reduced ODE model of the *PER/dCLOCK* system that includes intermediate steps to mimic delay effects without relying on DDEs, and we built upon it using SDEs to introduce biologically realistic fluctuations. We simulated and analyzed the system to identify the conditions under which sustained oscillations emerge and to explore how additional regulatory features influence robustness. Our approach provides a framework for understanding circadian rhythms and how they may fail under perturbations.

Methods

Model 1 (ODEs)

Equations

We modeled the core feedback loop between the *PER* protein and the *dCLOCK* transcription factor in *Drosophila melanogaster* using a system of ordinary differential equations (ODEs). The model is adapted from Smolen et al. (2002), who originally described the system using delay differential equations (DDEs). To improve numerical and analytical tractability, we reformulated their system using ODEs with manually implemented time delays via intermediate variables.

The full system of equations is as follows:

1. *PER Dynamics* $$\frac{d[PER]}{dt} = v_{sP}R_{sP} - k_{dP}[PER]$$

2. *PER Synthesis Activation Function* $$R_{sP} = \left(\frac{[dCLOCK_{free}]}{K_1 + [dCLOCK_{free}]}\right)\tau_1$$

3. $Free\ dCLOCK\ Definition$ $$[dCLOCK_{free}] = [dCLOCK] - [PER]$$

4. $dCLOCK\ Dynamics$ $$\frac{d[dCLOCK]}{dt} = v_{sC}R_{sC} - k_{dC}[dCLOCK]$$

5. $dCLOCK\ Inhibition\ Function$ $$R_{sC} = (\frac{K_2}{K_2 + [dCLOCK_{free}]})\tau_2$$

Table A. Variable and parameter definitions for Model 1's equations.

Symbol	Meaning	Units	Value
$[PER]$	Total concentration of *PER* protein	nM	—
$[dCLOCK]$	Total concentration of *dCLOCK* protein	nM	—
$[dCLOCK_{free}]$	Unbound (free) *dCLOCK* available to activate PER transcription	nM	—
v_{sP}	Max synthesis rate of *PER*	nM/hour	0.5
v_{sC}	Max synthesis rate of *dCLOCK*	nM/hour	0.25
k_{dP}	Degradation rate of *PER*	1/hour	0.5
k_{dC}	Degradation rate of *dCLOCK*	1/hour	0.5
K_1	Michaelis constant for *PER* synthesis activation	nM	0.3
K_2	Michaelis constant for *dCLOCK* synthesis inhibition	nM	0.1
τ_1	Time delay for *PER* synthesis (via *dCLOCK*)	hours	10
τ_2	Time delay for *dCLOCK* synthesis (via self-regulation)	hours	10

The biological meaning of each term is as follows:

- $v_{sP}R_{sP}$: *PER* synthesis is activated by free *dCLOCK* and saturates as $[dCLOCK_{free}]$ increases.
- $k_{dP}[PER]$**:** *PER* is degraded at a constant rate.
- $v_{sC}R_{sC}$: *dCLOCK* synthesis is inhibited by free *dCLOCK* (negative autoregulation).

- $k_{dC}[dCLOCK]$: *dCLOCK* degrades over time.
- Delays τ_1, τ_2: Capture transcriptional and translational lags essential for oscillatory behavior.

Parameter Selection
Parameter values were adapted from the model by Smolen et al. (2002), who selected values consistent with observed circadian behavior in *Drosophila*. We adopted these values directly because these parameters fall within physiological ranges used in other circadian rhythm studies and produce oscillations with a 24-hour period under appropriate conditions.

Numerical Simulation
We numerically solved the system of ODEs using the *scipy.integrate.odeint* solver in Python. This solver is well-suited for stiff biological systems and allows precise control over step sizes. To implement time delays, we used a custom delay-handling function, **get_delayed**, which retrieves state values from the simulation history at times $t-\tau$, approximating delay differential behavior in an ODE framework.

Initial Conditions: [PER] = 0.1 nM, [dCLOCK] = 0.1 nM

Time Range: Simulations ran for 120 hours (5 days) with a time step of 0.1 hours.

Model 2 (SDEs)
Equations

1. $PER\ Dynamics$ $\frac{d[PER]}{dt} = (v_{sP}R_{sP} - k_{dP}[PER])dt + \sigma_P\sqrt{[PER]} \cdot dW_P(t)$
2. $dCLOCK\ Dynamics$ $\frac{d[dCLOCK]}{dt} = (v_{sC}R_{sC} - k_{dC}[dCLOCK])dt + \sigma_C\sqrt{[dCLOCK]} \cdot dW_C(t)$

Table B. Variable and parameter definitions for Model 2's equations.

Symbol	Meaning	Units	Value
σ_C	Noise strength for PER	$\sqrt{nM^2/hour}$	0.02-0.3
σ_P	Noise strength for *dCLOCK*	$\sqrt{nM^2/hour}$	0.02-0.3
$dW(t)$	Wiener process increment (Brownian noise)	—	$\mathcal{N}(0,\ dt)$

The biological meaning of each term is as follows:

- $\sigma_P\sqrt{[PER]} \cdot dW_P(t)$: Introduces multiplicative noise into PER synthesis. The strength of fluctuations scales with the square root of PER concentration, capturing intrinsic noise from stochastic gene expression and molecular fluctuations.

- $\sigma_C \sqrt{[dCLOCK]} \cdot dW_C(t)$: Multiplicative noise in dCLOCK synthesis, also scaling with protein concentration. This reflects biological randomness in transcription/translation due to low molecule numbers.
- $dW(t) \sim \mathcal{N}(0, dt)$: A Wiener process increment, representing random fluctuations with mean zero and variance proportional to the time step.

Parameter Selection
We chose noise strengths between 0.02 and 0.3 based on established relationships between molecule count and intrinsic noise. As described by Tsimring (2014), the relative magnitude of intrinsic fluctuations in gene expression scales approximately as $\frac{1}{\sqrt{N}}$, where N is the number of protein molecules. Since Smolen et al. (2002) modeled PER and dCLOCK with typical molecule counts around 50–100, this corresponds to expected noise levels of about 10–20%. Our selected range captures this variability and allows us to test how different levels of noise affect the robustness of circadian oscillations.

Numerical Simulation
We implemented the SDE system using the Euler–Maruyama method, a standard scheme for numerically solving SDEs. This allowed us to simulate how noise impacts amplitude, phase stability, and period variability.

Initial Conditions: [PER] = 0.1 nM, [dCLOCK] = 0.1 nM

Time Range: Simulations ran for 120 hours (5 days) with a time step of 0.1 hours.

Code Set-Up
All simulations were conducted in a Jupyter notebook environment hosted on Google Colab using Python. We utilized the packages *numpy* for numerical computations, *scipy.integrate* for ODE solving, and *matplotlib* for data visualization.

Our code consists of four models:

- **model_full**: Defines the system of ODEs, incorporating delayed regulation of *PER* and *dCLOCK*.
- **simulate_ode_model**: Executes the stepwise integration loop, allowing dynamic access to prior time points for delay modeling.
- **get_delayed**: Helper function to retrieve historical concentrations from simulation memory buffers.
- **simulate_sde_model**: Simulates the SDE model using Euler–Maruyama.

Here is a link to the full code. The link is also available in the Appendix.

Results

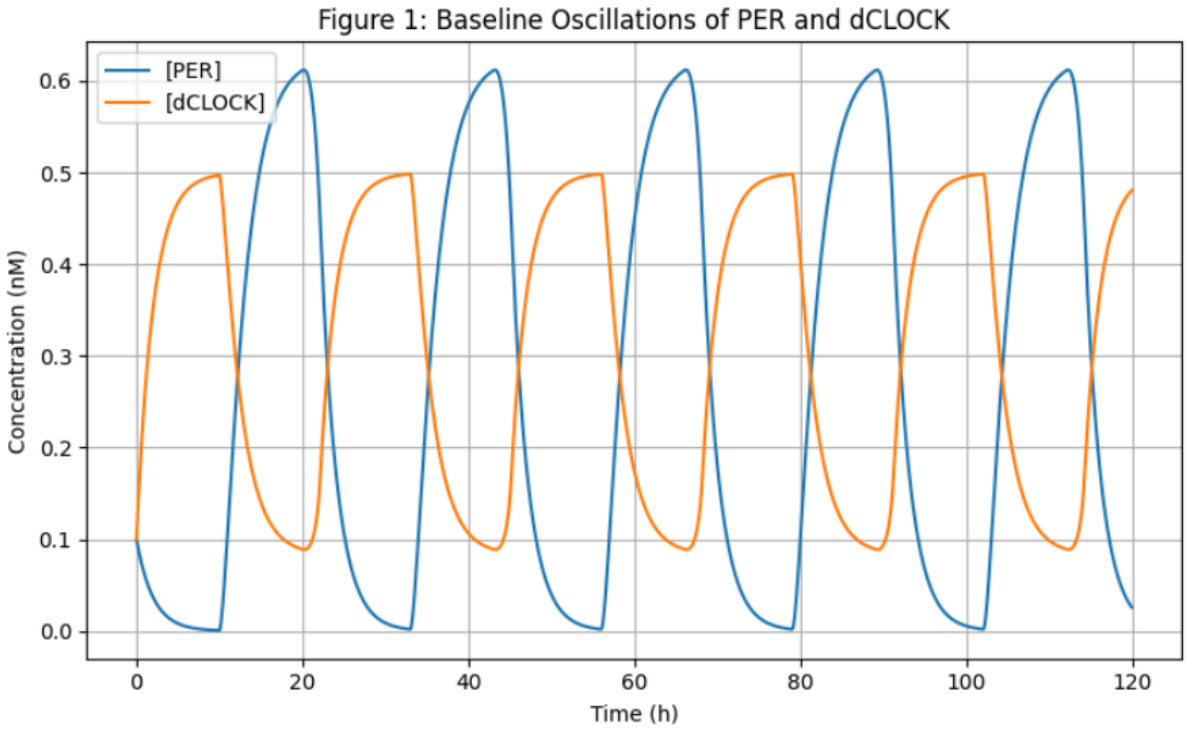

Figure 1. **Baseline Oscillations of PER and dCLOCK**
This figure shows the time evolution of *PER* (blue) and *dCLOCK* (orange) concentrations under baseline parameter values.

Both proteins exhibit sustained, periodic oscillations with a period of approximately 24 hours, closely mimicking biological circadian rhythms. The anti-phase oscillations of *PER* and *dCLOCK* proteins demonstrate that when *PER* concentration is high, *dCLOCK* is low, and vice versa. This inverse relationship supports a delayed negative feedback mechanism, where *PER* represses its own transcription by inhibiting *dCLOCK* activity. The presence of limit cycle oscillations (consistent amplitude and period without settling into a steady state) indicates a robust, self-sustaining rhythm.

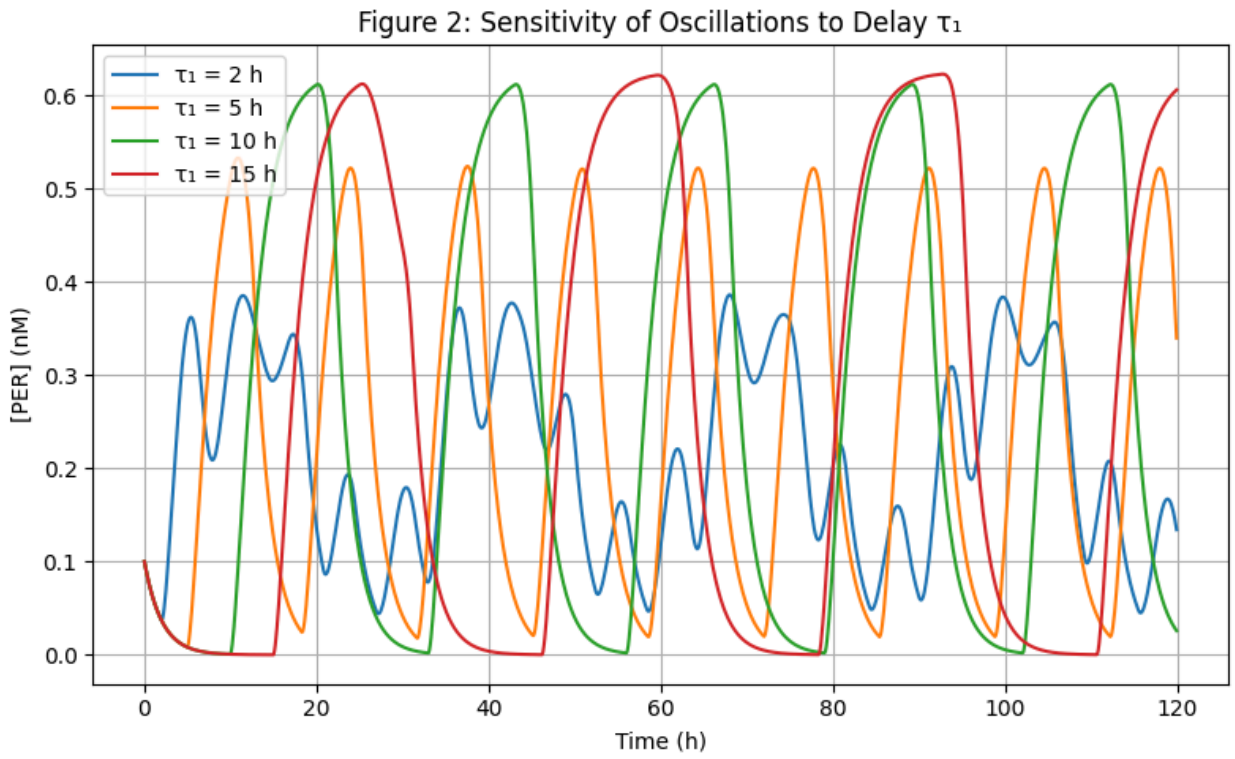

Figure 2. **Sensitivity of Oscillations to Delay** τ_1

The delay between changes in free *dCLOCK* and their effect on *PER* synthesis. Each line shows *PER* concentration over time for a different delay (τ_1 = 2, 5, 10, and 15 hours).

Varying the delay in negative feedback highlights a bifurcation. Short delays (2–5h) fail to support clean oscillations, leading to damped or irregular dynamics. However, beyond a threshold delay (~10h), the system exhibits sustained and periodic oscillations, with increasing sharpness and amplitude as the delay grows. This reveals that the system requires a minimum delay to accumulate enough *PER* to effectively repress *dCLOCK* after a lag, avoiding premature feedback that could destabilize oscillations.

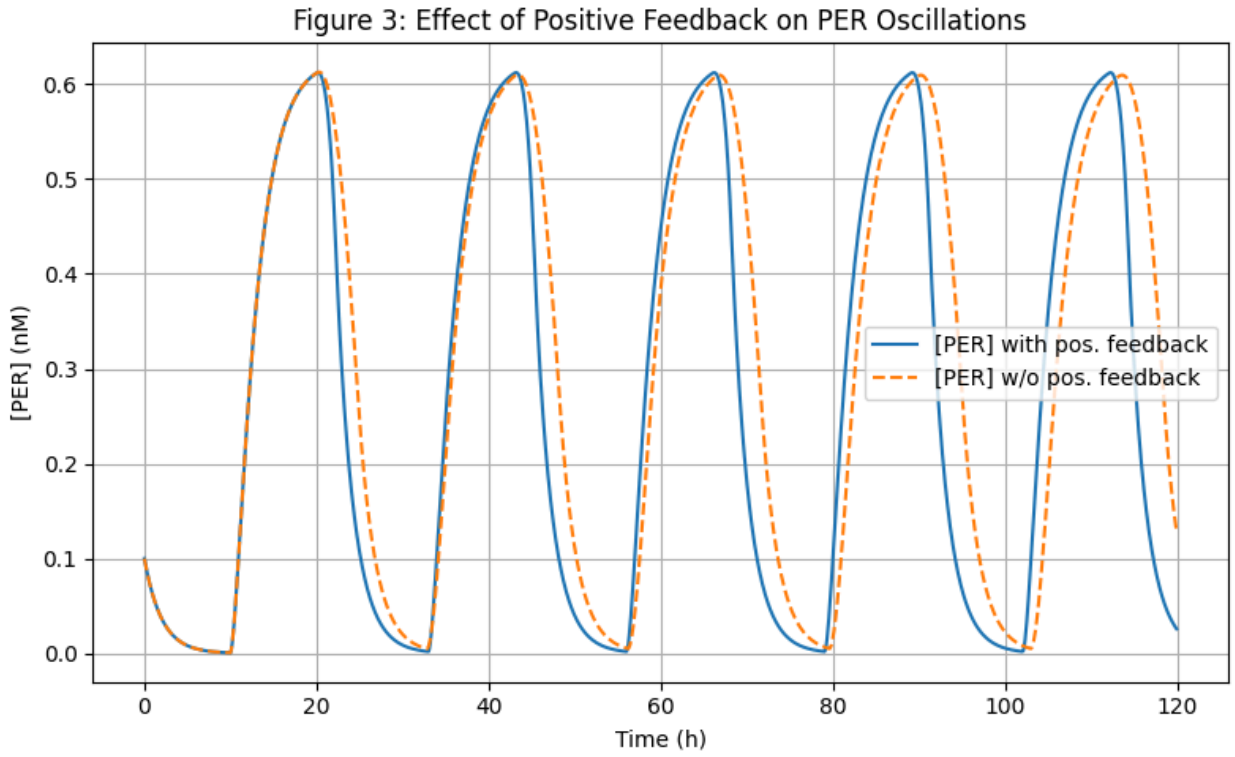

Figure 3. **Effect of Positive Feedback on PER Oscillations**
The full model (solid blue line) compared with a simplified version lacking *dCLOCK* self-repression (dashed orange line). In the simplified model, we hold $R_{sC} = 1$, removing positive feedback.

Here, both models produce oscillations with similar periods, showing that delayed negative feedback alone is sufficient to drive rhythmicity. However, the model lacking positive feedback *(dCLOCK* held constant) results in slightly lower amplitude and more symmetric oscillations. This suggests that positive feedback is not essential for rhythm generation but fine-tunes the system—enhancing amplitude, sharpening peaks, and potentially improving robustness.

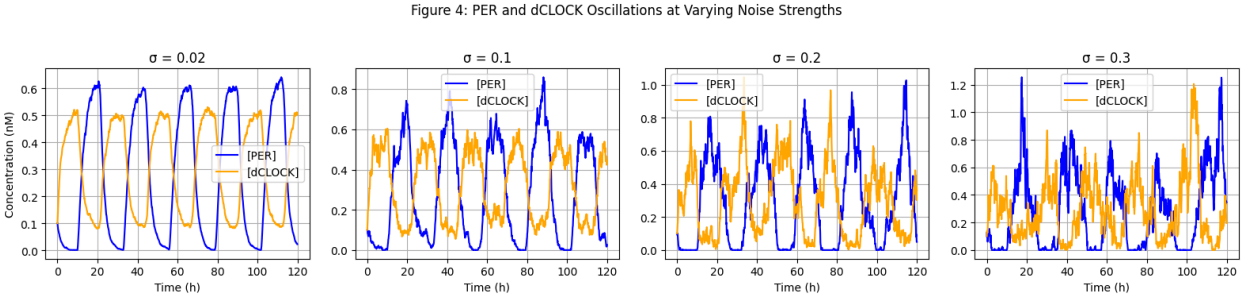

Figure 4. **Effect of Noise Strength on PER and dCLOCK Oscillations**
This figure shows the stochastic trajectories of PER (blue) and dCLOCK (orange) concentrations under increasing levels of intrinsic multiplicative noise: σ = 0.02, 0.1, 0.2, and 0.3. Each panel

represents a separate simulation where both PER and dCLOCK were subject to noise proportional to the square root of their own concentrations.

These results demonstrate that circadian oscillations are resilient to moderate levels of stochasticity, maintaining relatively stable amplitude and timing. However, as noise strength increases, the oscillations become increasingly irregular and less coherent. This suggests that while the system's delayed feedback and nonlinear regulation provide some buffering against fluctuations, there is a threshold beyond which noise significantly disrupts rhythm stability. These findings highlight the importance of noise-tolerant design in biological clocks to ensure reliable timekeeping under variable intracellular conditions.

Discussion

Overall, our model highlights delayed negative feedback as the core mechanism for circadian rhythm generation, with anti-phase *PER* and *dCLOCK* dynamics reflecting real biological behavior. Positive feedback fine-tunes the system by enhancing amplitude and robustness. Sustained oscillations emerge only when feedback delays exceed a threshold (~10h), showing that molecular delays are essential for rhythm stability. Positive feedback improves oscillation sharpness but has minimal effect on period. These results were mostly expected; the critical role of delay and the modulatory effect of positive feedback align with known circadian principles. Simulations across a range of noise strengths showed that the core oscillations remained relatively robust at low to moderate noise levels but became increasingly irregular at higher noise strengths.

Some limitations of our model include its simplified clock complexity (e.g., no *CRY, ROR* loops) and simplified *dCLOCK* dynamics. Moreover, our model assumed fixed delays in order to keep the model straightforward. If we were to redo this study in the future, we could try adding more regulatory loops (e.g., *CRY, REV-ERB*), including environmental cues, and using distributed delays to increase the model's complexity and realism.

Appendix: Code Repository

The code used in this study can be found at the following link: https://colab.research.google.com/drive/1zxnb2KNJcySJQUwrgO8r03DHGXXvgo2b?usp=sharing

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