Speaker Notes — Combined Presentation

Sensorimotor Habituation in Drosophila Larvae

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Slide 1: Title

Opening Thank you for having me. I will present work on sensorimotor habituation in Drosophila larvae, covering both our population-level modeling success and our subsequent attempt to extend the approach to individual phenotyping.

Transition The presentation has two parts. The first covers the original study where we developed a temporal kernel model. The second covers the follow-up study where we tested whether the same model could phenotype individual larvae.

Slide 2: Executive Summary — Original Study

Key Points to Emphasize - The gamma-difference kernel has **two timescales** that govern behavior - Fast excitation at tau1 ~ 0.3 seconds captures the initial sensory response - Slow suppression at tau2 ~ 4 seconds produces habituation across repeated stimuli - Model validated across 14 experiments and 701 unique tracks

Audience Anchor If there is one thing to remember from the original study, it is that larval reorientation dynamics can be captured by a simple parametric model with biologically interpretable timescales.

Slide 3: Kernel Structure

Figure Walkthrough - Left panel: The combined kernel showing the full temporal response - Right panel: Decomposition into fast gamma (green) and slow gamma (red) - The fast component peaks at 0.3 seconds and drives immediate response - The slow component peaks around 4 seconds and produces delayed suppression

Mathematical Point The kernel K(t) modulates reorientation hazard rate. Positive values increase turning probability. Negative values suppress it. The crossover from positive to negative creates the characteristic excitation-then-inhibition pattern.

Connection to Biology These timescales may correspond to distinct neural circuit mechanisms. The fast component could reflect direct sensory activation. The slow component could reflect adaptation or inhibitory feedback.

Slide 4: Simulated vs Empirical Event Counts

Validation Message Before using the model for anything, we need to confirm it generates realistic data. Panel A shows the histograms overlap well. Panel B shows the box plots match.

Key Numbers - 260 empirical tracks - 300 simulated tracks - Both show median around 15 events per track

Why This Matters The simulation framework is the foundation for power analysis. If simulations do not match empirical data, power calculations are meaningless.

Slide 5: Habituation Dynamics

Behavioral Phenomenon Turn fraction increases across LED pulses in all four experimental conditions. Larvae spend more time turning and less time running as the session progresses.

Condition Comparison - 0-250 Cycling shows the strongest habituation effect with slope +0.031 per pulse - 50-250 conditions show weaker effects - Shaded bands are 95% confidence intervals

Interpretation Habituation is the behavioral manifestation of the slow suppressive component accumulating across pulses. The kernel model predicts this effect.

Slide 6: Behavioral State Analysis

Detailed State Breakdown - Gray: Forward running - Pink: Turning - Blue: Pausing - Orange: Reverse crawling

Key Observation Turning fraction increases dramatically. Pausing remains below 5% throughout. Habituation manifests as increased turning, not increased pausing or freezing.

Quantitative Point By pulse 17 in the 50-250 Cycling condition, larvae spend nearly 40% of their time turning compared to about 20% at pulse 0.

Slide 7: LOEO Validation

What This Shows Leave-one-experiment-out cross-validation tests whether kernel parameters estimated from 13 experiments generalize to the held-out experiment.

Key Result Pass rate of 50% falls within the null distribution with $p = 0.618$. Cross-experiment generalization is no better than chance.

Interpretation The population model fits well overall, but individual experiments show high variability. This foreshadows the individual-level problems we will see in the follow-up study.

Transition This result motivated the follow-up question: Can we phenotype individual larvae using their unique kernel parameters?

Slide 8: Executive Summary — Follow-Up Study

Key Points to Emphasize - The answer to individual phenotyping is **negative** with current protocols - Sparse data with only 18-25 events per track makes 6-parameter estimation unreliable

- Apparent clusters are statistical artifacts of fitting high-dimensional models to low-event tracks - Only 8.6% of tracks show genuine individual differences

Audience Anchor The follow-up study is a negative result. We could not phenotype individuals. But the negative result is informative because it identifies the root cause and points toward solutions.

Slide 9: The Clustering Illusion

Figure Walkthrough - Panel A: PCA reveals unimodal distribution, not discrete clusters - Panel B: All four validation methods fail with ARI below 0.13 - Panel C: Gap statistic is minimized at k=1, indicating no clusters

Key Message K-means will always produce k clusters regardless of whether true clusters exist. The gap statistic tells us k=1 is optimal. There are no discrete phenotypes in this data.

Why It Matters Clusters identified by unsupervised learning are artifacts of sparse data, not genuine biological phenotypes. Publishing these clusters would be misleading.

Slide 10: Data Sparsity Explains Instability

The Math Problem - Mean 25 events per track - 6 kernel parameters to estimate - Data-to-parameter ratio is 4:1 - Reliable MLE requires at least 10:1

Visual Explanation Panel C shows the calculation: 4 parameters divided by 25 events equals a ratio of 6:1. This is fundamentally underdetermined.

Key Number 100 events per track is the target for stable estimation. Current protocols deliver only 25.

Slide 11: Hierarchical Shrinkage

What Shrinkage Does Bayesian hierarchical estimation pulls individual estimates toward the population mean. Tracks with sparse data shrink more. Tracks with abundant data retain their individual estimates.

Key Insight Shrinkage is not a bug. It is optimal regularization under the assumption that individuals are exchangeable members of a population.

Limitation Shrinkage cannot create information that is absent. With only 25 events, almost all individual estimates shrink heavily toward the population mean.

Slide 12: The Identifiability Problem

Figure Walkthrough - Panel A: Continuous design produces high bias and RMSE - Panel B: Burst design extracts 10× more Fisher Information per event - Panel C: MLE recovery differs dramatically by design - Panel D: Continuous fails because inhibition dominates during LED-ON

Key Insight The problem is not just data quantity but data quality. Continuous 10-second LED pulses produce events during the suppressive phase of the kernel. These events carry almost no information about tau1.

Recommendation Preview Switch to burst stimulation to sample the early excitatory window repeatedly.

Slide 13: Stimulation Protocol Comparison

Four Designs Shown - A: Current continuous 10s ON, 20s OFF - B: Recommended burst 10×0.5s with 2s spacing - C: Alternative 4×1s with 5s spacing - D: Alternative 2×2s with 10s spacing

Key Numbers Burst design provides 8× more Fisher Information than continuous. This could reduce the number of events required for reliable estimation from 100 to 30.

Slide 14: Kernel Model Comparison

Why Compare Models We chose the gamma-difference kernel for interpretability, but we need to verify it fits as well as flexible alternatives.

Results - Raised cosine basis: $R^2 = 0.974$ with 12 parameters - Gamma-difference: $R^2 = 0.968$ with 6 parameters

Interpretation The gamma-difference captures 96.8% of the variance explained by the flexible model with half the parameters. The timescales tau1 and tau2 are not just curve-fitting artifacts. They represent genuine temporal structure.

Slide 15: Recommendation 1 — Protocol Modification

Primary Recommendation Replace continuous 10-second ON periods with burst trains. Each burst event carries 10× more Fisher Information.

Quantitative Benefit This modification alone could reduce the number of events required for reliable estimation from 100 to approximately 30.

Implementation Change the LED control code to deliver 10 pulses of 0.5 seconds each with 2-second spacing instead of a single 10-second pulse.

Slide 16: Recommendation 2 — Extended Recording

Secondary Recommendation Target 40 minutes or more of recording to achieve at least 50 reorientation events per track.

Current State Current 10-20 minute recordings yield only 18-25 events.

Power Analysis Result 100 events are required for 80% power to detect a 0.2-second difference in tau1 at the individual level.

Slide 17: Recommendation 3 — Model Simplification

Approach Reduce the parameter space by fixing population-derived parameters.

Specific Suggestion - Fix tau2 at the population estimate of 3.8 seconds - Fix the amplitude ratio B/A at the population value - Estimate only the fast timescale tau1 per individual track

Rationale Hierarchical Bayesian estimation provides natural regularization toward the population mean. With only one free parameter, even 25 events may be sufficient.

Slide 18: Recommendation 4 — Alternative Phenotypes

Pragmatic Alternative Use robust composite phenotypes that avoid kernel fitting entirely.

Examples - ON/OFF event ratio: Measures whether larvae respond preferentially during LED-ON versus LED-OFF - First-event latency: Time from LED onset to first reorientation

Advantage These phenotypes require only event counts, not full 6-parameter kernel estimation.

Slide 19: Recommendation 5 — Within-Condition Analysis

Methodological Point Analyze individual differences within experimental conditions rather than pooling across conditions.

Why This Matters When data from different stimulation intensities and temporal patterns are pooled, condition effects dominate and mask genuine individual variation.

Evidence The ARI near zero across all validation methods indicates no reproducible structure when pooling.

Slide 20: Conclusions — Original Study

Summary of Success - Gamma-difference kernel accurately models population-level dynamics - Two timescales govern behavior: tau1 $\sim$ 0.3s for excitation, tau2 $\sim$ 4s for suppression - Model is robust across experimental conditions - Biological interpretability with equivalent goodness of fit

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Slide 21: Conclusions — Follow-Up Study

Summary of Challenge - Individual phenotyping fails with current protocols due to sparse data - Apparent clusters are statistical artifacts - Only 8.6% of tracks show individual variation exceeding noise - Current protocols achieve only 20-30% statistical power

Bottom Line Population-level analysis is robust and biologically meaningful. Individual phenotyping requires experimental redesign before kernel-based classification becomes reliable.

Slide 22: Thank You

Transition to Questions I am happy to take questions. For common questions, I have prepared some FAQ slides.

Slides 23-27: FAQ

Prepared Answers

Q: What is the sequence of processes in the original study? Data collection → MAGAT trajectory extraction → Event detection → Population kernel fitting → LOEO validation

Q: What processes were used in the follow-up study? Individual MLE fitting → K-means/hierarchical clustering → Round-trip validation → Power analysis → Identifiability analysis

Q: Why does population modeling succeed but individual fails? Data-to-parameter ratio. Population pools ~15,000 events for 6 parameters (2500:1). Individual uses ~25 events for 6 parameters (4:1).

Q: What is hierarchical shrinkage? Bayesian regularization that pulls individual estimates toward the population mean proportionally to data sparsity.

Q: How should clustering results be interpreted? With extreme skepticism. K-means will always produce k clusters. The gap statistic shows k=1 is optimal. Round-trip validation shows ARI < 0.2.

Anticipated Questions and Answers

Q: Could a different kernel form work better for individual phenotyping? A: Unlikely. The problem is data quantity and quality, not kernel form. Simpler models like single-timescale exponentials might help by reducing parameters.

Q: What about using machine learning instead of kernel fitting? A: ML methods face the same fundamental problem. With 25 events per track, there is insufficient information to distinguish individuals regardless of the algorithm.

Q: How confident are you in the 100-event threshold? A: The 100-event threshold comes from simulation-based power analysis targeting 80% power for a 0.2-second effect. Different effect sizes would require different thresholds.

Q: Are there any individual larvae that do show reliable phenotypes? A: Yes, 8.6% of tracks show individual variation exceeding measurement noise. These are the "outliers" that retain individual estimates after hierarchical shrinkage. But 8.6% is too few for systematic phenotyping.

Q: What is the next step for this research? A: Implement burst stimulation protocol and collect new data with 40+ minute recordings. Rerun the phenotyping analysis with the improved data.

Timing Guide

Slides	Section	Target Time
1-2	Introduction	2 min
3-7	Original Study	8 min
8-14	Follow-Up Study	10 min
15-19	Recommendations	5 min
20-22	Conclusions	3 min
23-27	FAQ (if needed)	5 min

Total: 28-33 minutes

Technical Terms to Define if Asked

Gamma-difference kernel: Difference of two gamma distributions, one fast (excitatory) and one slow (suppressive)

PSTH: Peri-stimulus time histogram, the empirical distribution of event times relative to stimulus onset

Fisher Information: Measure of how much information an observable contains about an unknown parameter

Hierarchical shrinkage: Bayesian regularization toward population mean

Gap statistic: Method for determining optimal number of clusters by comparing within-cluster dispersion to null reference

ARI: Adjusted Rand Index, measure of agreement between two clusterings corrected for chance

MLE: Maximum likelihood estimation

LOEO: Leave-one-experiment-out cross-validation