Associative Learning and Conflict Resolution in a Cross-Species Hybrid Nervous System

Hybrid #001 Technical Report 3

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Abstract

We report results from Experiments 3 and 4 of the Hybrid #001 digital nervous system. In Experiment 3 (Associative Learning), we pair a chemotactic stimulus with a dopaminergic reward signal over 50 conditioning trials and test for conditioned motor responses. We find that the hybrid develops a progressive increase in forward motor bias to the conditioned stimulus, reaching a 41% increase in selectivity ratio by trial 50 compared to trial 1, while the worm-only system shows no significant change across trials ($p = 0.72$). The learning curve follows an exponential saturation consistent with dopamine-gated Hebbian plasticity at KC-MBON synapses. In Experiment 4 (Cross-Species Conflict), we simultaneously activate nociceptive (reversal-promoting) and reward (approach-promoting) pathways. The hybrid produces a novel motor pattern: delayed, attenuated forward locomotion with intermittent pauses, a response absent from both the worm-only system (which reverses) and the fly-only system (which shows sustained approach-biased output). This conflict resolution behavior constitutes the first evidence of emergent cross-species integration producing behavior generated by neither parent system alone.

Keywords: associative conditioning, dopamine-gated plasticity, conflict resolution, emergent behavior, cross-species neural integration, Kenyon cells, MBON

1. Introduction

Technical Report 2 established that the bridge layer of Hybrid #001 produces functionally meaningful signal translation between *C. elegans* and *Drosophila* neural circuits, with temporally structured cross-

species correlations and a 34% increase in sensorimotor selectivity. Those results demonstrated signal propagation but not adaptive behavior. This report presents the critical experiments: does the hybrid learn, and does it resolve conflicting drives in ways neither parent system can?

The biological basis for these experiments lies in the complementary capacities of the two parent systems. *C. elegans* exhibits robust sensorimotor reflexes but limited associative plasticity. *Drosophila*'s mushroom body implements a well-characterized form of associative learning: dopaminergic neurons (DANs) gate Hebbian plasticity at Kenyon cell-to-MBON synapses, enabling conditioned approach and avoidance responses (Aso et al., 2014; Hige et al., 2015). The bridge layer routes worm sensory signals into fly Kenyon cells and fly reward signals back to worm modulatory neurons, creating the substrate for cross-species conditioning.

2. Methods

2.1 Experiment 3: Associative Learning

The conditioning protocol consisted of 50 paired trials, each 1,000 ms in duration. On each trial, the chemotactic stimulus pathway (ASEL, ASER, AWCL, AWCR) was activated at t = 100 ms with a 30 mV current injection, followed 200 ms later by a reward signal (DAN2 activation, 25 mV) at t = 300 ms. The 200 ms CS-US interval is consistent with effective conditioning windows in *Drosophila* olfactory learning (Tully & Quinn, 1985). Inter-trial interval was 2,000 ms to allow activity to return to baseline.

After conditioning, 20 test trials presented the chemotactic stimulus alone (no DAN2 activation). Motor selectivity ratio was measured as in Experiment 2. To implement synaptic plasticity, a Hebbian update rule was applied at KC-MBON synapses: when a Kenyon cell and its downstream MBON were co-active within a 50 ms window during DAN2 activation, the KC-MBON weight was incremented by 0.05 (additive, capped at 2x baseline). This follows the DAN-gated plasticity model of Hige et al. (2015).

2.2 Experiment 4: Cross-Species Conflict

Nociceptive (ASHL, ASHR; 35 mV, promoting reversal via AVAL/AVAR) and reward (DAN2; 25 mV, promoting approach via MBON-CX-DN-BR_DM-AVBL pathway) signals were activated simultaneously at t = 0. Motor neuron spike counts were recorded over a 500 ms window. The experiment was run in three configurations: worm-only (nociception only, as DAN2 does not exist), fly-only (reward only, as ASHL/ASHR do not exist), and hybrid (both stimuli, full bridge). Five additional control conditions were tested: (1) hybrid with bridge disconnected, (2) hybrid with BR_DM only, (3) hybrid with BR_RW only, (4) nociception alone in hybrid, (5) reward alone in hybrid.

3. Results

3.1 Associative Learning

The hybrid system showed progressive acquisition of conditioned motor bias across 50 training trials. The forward/reverse selectivity ratio increased from $S = 2.3 \pm 0.5$ on trial 1 to $S = 3.2 \pm 0.7$ on trial 50, a 41% increase. The learning curve was well-fit by an exponential saturation function $S(n) = S_{\max}(1 - e^{-n/\tau}) + S_0$, with time constant $\tau = 14.2$ trials and asymptote $S_{\max} = 3.4$ ($R^2 = 0.91$). This time constant is consistent with the rate of KC-MBON synaptic modification under the implemented plasticity rule.

The worm-only system showed no significant change in selectivity across 50 trials ($S = 2.1 \pm 0.4$ on trial 1, $S = 2.0 \pm 0.5$ on trial 50; $p = 0.72$, Wilcoxon signed-rank test). This confirms that the learning effect in the hybrid is mediated by the fly's associative circuits, not by any inherent plasticity in the worm subsystem.

Table 1. Selectivity ratio across conditioning blocks.

Trial block	Worm-only S	Hybrid S	p (hybrid vs block 1)
1–10	2.1 ± 0.4	2.4 ± 0.5	0.31 (n.s.)
11–20	2.0 ± 0.5	2.7 ± 0.5	0.04
21–30	2.1 ± 0.4	2.9 ± 0.6	0.008
31–40	2.0 ± 0.5	3.1 ± 0.6	0.002
41–50	2.1 ± 0.4	3.2 ± 0.7	< 0.001

On the 20 post-conditioning test trials (chemotactic stimulus alone, no reward), the hybrid maintained an elevated selectivity ratio of $S = 2.9 \pm 0.6$, significantly above pre-conditioning baseline ($p < 0.01$). The conditioned response decayed slowly over the test block (from 3.1 to 2.7), consistent with extinction dynamics in biological conditioning paradigms.

3.2 KC-MBON Weight Changes

Analysis of synaptic weights after conditioning revealed selective strengthening of KC-MBON synapses that were co-active during reward delivery. KC1-MBON1 weight increased from 2.0 to 3.2 (60% increase), KC2-MBON2 from 2.0 to 2.8 (40%), and KC3-MBON3 from 2.5 to 3.6 (44%). Kenyon cells

KC4 and KC5, which receive weaker bridge input and were less consistently co-active with the reward signal, showed minimal weight changes (KC4-MBON1: 1.5 to 1.7; KC5-MBON4: 2.0 to 2.1). This selective strengthening pattern is consistent with the sparse coding principle: only the subset of Kenyon cells activated by the specific sensory context undergo plasticity.

3.3 Cross-Species Conflict

Simultaneous nociceptive and reward activation produced qualitatively different motor patterns across configurations:

Table 2. Motor output during conflict condition (500 ms window).

Configuration	Forward spikes	Reverse spikes	*S* ratio	Pattern
Worm-only (nociception)	2.1 ± 0.8	8.7 ± 1.4	0.24	strong reversal
Fly-only (reward)	—	—	—	sustained MBON/DN output
Hybrid (conflict)	4.3 ± 1.2	5.1 ± 1.5	0.84	attenuated, intermittent
Hybrid (bridge disconnected)	2.0 ± 0.9	8.5 ± 1.3	0.24	pure reversal (= worm-only)
Hybrid (nociception alone)	2.3 ± 0.7	7.9 ± 1.5	0.29	reversal
Hybrid (reward alone)	6.8 ± 1.3	2.4 ± 0.6	2.83	forward bias

The hybrid under conflict conditions produced a selectivity ratio of $S = 0.84$, intermediate between the strong reversal of worm-only ($S = 0.24$) and the strong forward bias of hybrid-reward-alone ($S = 2.83$). Critically, the hybrid conflict response was not a simple average of the two drives. Analysis of the temporal motor pattern revealed a distinctive signature: an initial 80 ms pause (neither forward nor reverse spikes), followed by attenuated forward motor activity with intermittent 20–40 ms gaps. This pattern was absent from all control conditions.

3.4 Bridge Neuron Dynamics During Conflict

During the conflict condition, bridge neurons showed elevated and irregular firing patterns. BR_DM fired at 12.4 Hz (vs 7.1 Hz baseline), driven by DN1/DN2 output from the fly reward pathway. BR_RW fired at 8.9 Hz, relaying the fly reward signal to worm modulatory neurons RIML/RIMR. Simultaneously, AVAL (reverse command) received strong excitatory input from the nociceptive pathway via AIBL/AIBR. The competition between AVAL excitation (nociception) and AVAL inhibition (BR_DM) produced the characteristic pausing behavior: during periods when BR_DM inhibitory drive temporarily matched nociceptive excitatory drive, AVAL firing was suppressed without AVBL being strongly driven, resulting in motor silence.

This competitive inhibition mechanism is novel. In the worm-only system, nociception drives reversal without opposition. In the hybrid, the bridge injects a competing signal from a different species' decision-making circuits. The result is not paralysis but a modulated response that preserves elements of both drives: the organism pauses (acknowledging the nociceptive threat), then moves forward cautiously (driven by the reward signal). This is a form of behavioral compromise that emerges from the hybrid architecture.

4. Discussion

These experiments provide two lines of evidence for the compositional modularity hypothesis:

First, the hybrid learns. The 41% increase in conditioned motor selectivity over 50 trials, mediated by DAN-gated Hebbian plasticity at KC-MBON synapses, demonstrates that the fly's associative learning circuits can be driven by worm sensory signals routed through the bridge and can produce lasting changes in worm motor output. This is cross-species associative conditioning: the worm's sensors detect the stimulus, the fly's mushroom body learns the association, and the worm's motors execute the conditioned response. No single species' circuits perform this complete sequence alone.

Second, the hybrid resolves conflicts. When simultaneously threatened (nociception) and rewarded (approach signal), the hybrid produces a motor pattern absent from either parent system: a cautious, intermittent forward movement preceded by a pause. This behavior satisfies our criterion for emergence: it is generated by neither parent system alone, it requires the bridge layer (disconnecting the bridge produces pure reversal), and its temporal structure (pause-then-cautious-approach) has a coherent behavioral interpretation.

We note two important caveats. First, the implemented plasticity rule is simplified relative to biological KC-MBON dynamics, which involve compartmentalized dopamine modulation with distinct roles for appetitive (PAM) and aversive (PPL1) signaling (Aso et al., 2014). Our model uses a single reward signal (DAN2/PAM) and does not distinguish between approach and avoidance learning. Second, the conflict resolution behavior, while qualitatively novel, has not been tested for robustness across parameter variations. A systematic sensitivity analysis is required to establish whether the pause-then-approach pattern is a robust feature of the architecture or an artifact of specific parameter choices.

5. Conclusion

The Hybrid #001 digital nervous system exhibits two forms of adaptive behavior absent from either parent system: cross-species associative learning and conflict resolution. These results support the hypothesis that neural circuit motifs are composable across species boundaries, provided that the inter-species interface (bridge layer) performs structured signal translation rather than simple relay. The four bridge neurons translate between worm and fly signal formats with sufficient fidelity to support both tonic modulation (Report 2), progressive learning (this report), and real-time competitive integration of conflicting drives (this report).

Whether these results generalize beyond this specific species pair and circuit selection remains an open question. As additional complete connectomes become available (zebrafish, mouse cortical columns), the bridge layer formalism introduced here provides a framework for testing cross-species composability at larger scales.

References

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