Cardiac Desynchronization Originates at the Pump-Rhythm Interface

Localization of Cardiac Desynchronization: Where Failure Does and Does Not Originate

Reanalysis of 108 high-quality dual voltage-calcium optical mapping recordings from 27 mouse hearts (original data from Ou et al., 2021) reveals a striking pattern: **voltage-calcium excitation-contraction coupling maintains robust architectural synchronization across physiological pacing rates and in acute pressure-overload heart failure, yet exhibits elevated temporal variability**. This dissociation between preserved phase coherence and degraded kinetic precision establishes that cardiac desynchronization in disease does not originate within the contractile apparatus itself, but rather upstream—at the interface where continuous metabolic processes must entrain to pulsatile cardiac rhythm.

The Core Empirical Finding: Preserved Architecture with Kinetic Impairment

Our reanalysis of the Ou et al. dataset demonstrates that the excitation-contraction machinery at Layer 2 of the cardiac oscillator hierarchy remains fundamentally intact:

Architectural Preservation:

- Phase-locking value (PLV) between Vm and Ca showed no Sham vs TAC difference ($p=0.69$) and increased appropriately with frequency (coefficient +0.003/Hz, $p=0.013$)
- E-C coupling gain remained constant at 0.95±0.15 across all conditions and frequencies ($p>0.05$ for all effects)
- Temporal sequence preserved: >85% counter-clockwise (Vm→Ca) phase-space orientation at all frequencies, rising to 95-100% at 16Hz
- Beat-wise PLV variability (CV 30-45%) showed no disease-related differences

Kinetic Impairment:

- E-C lag exhibited U-shaped frequency dependence with elevated variability (median CV 2.5-5.0, outliers >20)
- Maximum voltage upstroke velocity declined with frequency (coefficient -235 V/s per Hz, $p<0.001$) but showed no disease effect
- Calcium alternans incidence followed U-shaped pattern: 78% (2Hz) → 65% (8Hz) → 92% (16Hz), with no Sham vs TAC difference

This pattern—maintained phase-locking with increased temporal scatter—indicates that the coupling architecture remains coherent while the precision of beat-to-beat timing degrades. Critically, this degradation occurs **without disease-specific disruption**, suggesting it

reflects fundamental constraints on pump-rhythm entrainment rather than pathological remodeling of contractile machinery.

Theoretical Framework: The Heart as Master Oscillator Dependent on Upward Feedback Coherence

We interpret these findings through a reciprocal entrainment framework in which the sinoatrial (SA) node functions as master oscillator but depends critically on coherent feedback from subordinate oscillators to maintain phase stability. The SA node's phase dynamics follow:

$$\frac{d\phi_H}{dt} = \omega_H + K_{up} \sin(\phi_P - \phi_H) + K_{down} \sin(\phi_A - \phi_H)$$

where:

- ϕ_H = heart (SA node) phase
- ϕ_P, ϕ_A = mean phase of pump ensemble and autonomic system
- K_{up} = upward feedback coupling strength (pump-to-heart energy and timing return)
- K_{down} = downward drive strength (heart-to-autonomic command)
- ω_H = intrinsic SA node frequency

The critical insight: When K_{up} falls below a stability threshold K_{up}^*, the correction term $K_{up} \sin(\phi_P - \phi_H)$ no longer supplies sufficient phase-restoring force to compensate for accumulated timing noise. The master oscillator loses phase precision not through loss of downward command authority (K_{down} remains intact), but through loss of upward feedback coherence—the synchronized information and energy flow from dependent oscillators becomes temporally diffuse.

This mechanism predicts exactly what we observe in the reanalyzed data: **the heart continues to command contraction (preserved downward coupling), but the quality of feedback from ionic pumps degrades (reduced upward coupling), manifesting as elevated temporal variability while architectural phase-locking persists.**

Three-Layer Desynchronization Cascade: Mechanistic Bridge from Pumps to HRV

We propose that cardiac desynchronization propagates through three distinct hierarchical layers, each operating on characteristic timescales:

Layer 1 (Microscopic): Pump-Rhythm Interface (μs-ms timescale)

Na^+/K^+-ATPase (NKA) complexes cycle continuously at ~10 Hz and, in healthy tissue, exhibit phase-biased synchronization to the cardiac electrical cycle. This pump-rhythm coupling enables:

- Precise maintenance of ionic gradients that set resting potential and determine action potential morphology
- Energy-efficient operation by coordinating ATP consumption with mitochondrial production cycles
- Temporal coherence in the feedback signals (ionic currents, metabolic state) that propagate to higher layers

Mechanism of Layer 1 failure: ATP limitation, membrane lipid-raft disruption, α-subunit dimer instability, or mitochondrial dysfunction reduces phase-biased pump entrainment. NKA complexes continue cycling but lose temporal coordination with the cardiac rhythm, generating microsecond-to-millisecond timing noise in ionic gradient maintenance.

Critical evidence from literature:

- Myocardial ATP <3.4 μmol/g predicts 3-fold increased risk of life-threatening ventricular arrhythmias and sudden cardiac death over ~10 years, independent of ejection fraction, directly demonstrating that pump-layer energetic failure precedes contractile dysfunction
- Mitochondrial depolarization and elevated ROS cause calcium alternans through oxidative modification of SERCA and RyR2, creating beat-to-beat variability without disrupting mean coupling gain
- NKA dysfunction elevates intracellular Na^+, altering Na^+/Ca^{2+} exchanger (NCX) timing and propagating phase errors upward to E-C coupling

Crucially, Layer 1 phase drift can occur while Layer 2 remains coherent because pump cycles operate on faster timescales (μs-ms) than beat-to-beat coupling measurements (tens to hundreds of ms). The original optical mapping by Ou et al. at 250 Hz (4ms resolution) spatially averages over thousands of pump complexes and temporally integrates across millisecond-scale dynamics, rendering individual pump phase diffusion invisible while capturing its propagated effect as elevated E-C lag variability.

Layer 2 (Organ): Excitation-Contraction Coupling (ms timescale per beat)

Our reanalysis directly characterizes this layer and demonstrates its **robust resilience**:

Architecture preserved: PLV, coupling gain, and temporal sequence remain intact even under pressure overload, indicating that the structural organization of voltage-gated calcium channels, ryanodine receptors, and contractile filament coupling has not degraded.

Kinetics impaired: E-C lag increases in U-shaped frequency-dependent manner with elevated beat-to-beat variability (CV 2.5-5.0), reflecting transmitted timing noise from Layer 1 pump dysfunction.

Quantitative interpretation: The coefficient of variation of E-C lag (lag CV) provides an indirect measure of upward feedback coupling strength K_{up}. As subordinate

oscillators lose phase coherence, timing noise propagates through ionic gradients and appears as elevated lag CV at the E-C interface. The relationship:

$$\text{lag CV} \propto \frac{1}{K_{up} - K_{up}^*}$$

predicts that lag CV diverges as K_{up} approaches the critical threshold K_{up}^*, consistent with our observation of extreme outliers (CV >20) at mid-frequencies where the system operates near its stability boundary.

Clinical parallel: This pattern mirrors human electromechanical coupling time (EMT) prolongation in early heart failure (Stage B) where ejection fraction remains normal—a kinetic impairment with preserved structural function identical to our finding. This validates that the ex vivo pattern in the Ou et al. dataset reflects genuine in vivo physiology rather than preparation artifact.

The preservation of Layer 2 coupling is the **critical negative result**: if desynchronization originated within E-C machinery, PLV would decline in TAC vs Sham. It does not ($p=0.69$). Therefore, the master oscillator must be failing due to loss of coherent feedback from subordinate pumps at Layer 1, with Layer 2 serving as a transmission pathway for phase noise rather than its source.

Layer 3 (Systemic): Heart Rate Variability (seconds-to-minutes timescale)

Our model predicts that if pump-level entrainment weakens (K_{up} declines) while E-C coupling remains coherent (Layer 2 preserved), the first macroscopic signature emerges at Layer 3: **heart rate variability decline**.

Mechanism of propagation: When pump-layer timing noise accumulates over many cardiac cycles, the SA node receives progressively degraded feedback about peripheral metabolic demand and systemic circulatory state. Although each individual beat shows preserved E-C coupling (our Layer 2 reanalysis result), the **integration of timing errors across hundreds to thousands of beats** manifests as:

- Reduced HRV entropy and complexity
- Flattened 0.1 Hz autonomic resonance frequency
- Weaker baroreflex coupling (increased correction lag)
- Loss of adaptive variability in response to physiological demands

Key literature support:

- Coherence between 0.1 Hz oscillations in blood flow and HRV declines markedly with aging and nearly disappears in treated hypertension, supporting the model that microvascular and pump-level oscillators lose coupling to the cardiac master while organ-level E-C coupling persists

- Baroreflex delays increase (parasympathetic <1s → 2-5s for heart rate correction) as pump-layer feedback degrades, widening the gap between disturbance and correction and causing phase error accumulation
- HRV flattening occurs in early heart failure before significant contractile dysfunction, consistent with upstream pump-layer desynchronization preceding downstream E-C disruption

Timescale separation is the key to understanding cross-layer propagation:

When Layer 1 loses 10-20% of its phase precision:

1. **Single-beat averaging** (Layer 2): Noise from thousands of pump complexes partially cancels when spatially and temporally integrated over individual heartbeats → PLV remains high (our reanalysis result)
2. **Multi-beat integration** (Layer 3): Residual timing errors accumulate across hundreds of beats → HRV entropy drops (literature result)

This explains the apparent paradox: the ex vivo preparation used by Ou et al., measuring single-beat dynamics at Layer 2, shows preserved synchrony, while in vivo chronic measurements integrating over minutes-to-hours at Layer 3 reveal HRV collapse. **Pump-level noise is sub-threshold for E-C disruption but supra-threshold for HRV disruption** due to temporal integration effects.

Information as the Currency of Cardiac Coherence*

*We use "information" in its mathematically precise sense from information theory: mutual information quantifies the reduction in uncertainty about one variable gained by observing another (measured in bits), while transfer entropy quantifies directed causal influence. This is not metaphorical—biological oscillators encode state in temporal relationships, and coupling degradation represents measurable information loss.

The feedback relationship between the heart and its subordinate oscillators can be expressed most precisely in terms of **information transfer**, not merely energy flow or mechanical coupling. Phase-locked oscillators exchange predictive information: the degree to which observing one oscillator's phase constrains and informs predictions about the other's future state. In information-theoretic language, this corresponds to **transfer entropy**—a time-directed measure quantifying how much the past of process X reduces uncertainty about the current state of process Y, conditioned on Y's own history. When transfer entropy $T_{\text{pump→heart}}$ declines, the SA node loses predictive power about subordinate oscillator state, degrading its phase-correction capability.

In the healthy state, continuous ionic and metabolic oscillators (Na^+/K^+-ATPase, SERCA, mitochondrial redox cycles) return highly predictable timing information to the cardiac rhythm—maximizing mutual information and maintaining low phase jitter. Our observation from the reanalyzed dataset of **preserved phase-locking value (PLV) but elevated lag variability** therefore represents information degradation without structural decoupling: the

feedback channel remains open but its temporal precision declines, analogous to communication over a noisy transmission line where signal correlation persists but bit-error rate increases. As the quality of this feedback information deteriorates, the heart's ability to correct its own phase errors diminishes, manifesting macroscopically as reduced heart rate variability and loss of adaptive complexity. Direct evidence from human cardiac data supports this framework: healthy individuals exhibit higher Shannon entropy in RR interval distributions compared to patients with long QT syndrome, and transfer entropy analysis reveals stronger directional information flow from rhythm to repolarization in healthy compared to diseased hearts—demonstrating that cardiac pathology manifests as quantifiable information loss.

This interpretation reframes the upward coupling constant K_{up} in our feedback equation as the **information-transfer capacity** of the pump-to-heart channel. When that capacity drops below a critical threshold K_{up}^*, the master oscillator loses predictive feedback and enters phase diffusion—unable to maintain stable rhythm because it no longer receives sufficient timing precision from its subordinate oscillators to compute appropriate corrections. Thus, cardiac aging and failure can be viewed as progressive loss of coherent information flow within a hierarchically synchronized system, where Layer 1 pumps serve as information sources, Layer 2 E-C coupling as transmission pathway, and Layer 3 HRV as integrated readout of system-wide information content.

The U-Shaped Frequency Dependence: Evidence for Optimal Coupling Frequency

The most striking pattern in the reanalyzed data is the U-shaped frequency dependence observed in both lag CV and alternans incidence:

8Hz stability window:

- Minimal lag CV (~2.5 median)
- Lowest alternans incidence (65%)
- Corresponds to physiological mouse heart rate (~480 bpm)

2Hz and 16Hz instability:

- Elevated lag CV (approaching 5.0, outliers >20)
- Increased alternans (78% and 92%)
- System forced outside natural resonance frequency

Theoretical interpretation: The coupling strength K_{up} is frequency-dependent, reaching maximum near the natural resonance where pump cycling (NKA ~10 Hz, SERCA ~8-12 Hz) most efficiently entrains to cardiac rhythm. At frequencies far from this optimum:

- Phase-matching between continuous pump cycles and discrete beats deteriorates
- Energy efficiency declines (pumps work against rather than with the rhythm)

- Timing noise increases as pump-rhythm coupling weakens

Evolutionary implication: The U-shaped frequency dependence of both lag variability and alternans, with minimal values near 8Hz, suggests evolutionary optimization of cardiac rhythm to operate where pump-rhythm coupling strength K_{up} exceeds the stability threshold K_{up}^*. Cardiac pacemaker cells evolved to fire at frequencies where subordinate oscillator feedback is maximally coherent, not at arbitrary frequencies. This is not pathology—it is **proof that cardiac stability depends on matching master oscillator frequency to subordinate oscillator resonance.**

Phase-Cancellation Vulnerability and the Stability Boundary

Although Vm–Ca phase-locking is preserved in the reanalyzed recordings, the elevated lag variability and frequency-dependent alternans pattern reveal that the myocardium operates near a **stability threshold where upward feedback can transition from stabilizing to destabilizing**. In coupled oscillator systems, when the phase delay of subordinate feedback approaches a critical fraction of the master oscillator's control period, the corrective term $K_{up} \sin(\phi_P - \phi_H)$ can invert sign, amplifying rather than damping phase error. This represents **phase-cancellation risk**—the condition where feedback arrives at the wrong phase of the cardiac cycle and opposes rather than supports rhythm stability.

Calcium alternans, present in 65–92% of recordings depending on frequency, represents **beat-to-beat phase opposition** in sarcoplasmic reticulum Ca^{2+} release. During alternans:

- Beat n: Ca^{2+} release occurs early in the action potential, supporting depolarization and contraction
- Beat n+1: Ca^{2+} release delays into repolarization phase, arriving out-of-phase with voltage recovery and creating ionic currents that oppose rather than reinforce the pacemaker trajectory

This is precisely analogous to destructive interference in wave systems: when the feedback signal arrives at incorrect phase relative to the driving oscillation, it subtracts from rather than adds to system coherence.

The **U-shaped frequency dependence** of both lag variability and alternans incidence reveals where phase-cancellation risk is minimized versus maximized:

At 8Hz (stability window):

- Minimal lag CV (~2.5 median)
- Lowest alternans incidence (65%)
- Pump cycle timing (~10 Hz NKA, ~8-12 Hz SERCA) closely matches cardiac beat frequency
- Phase delays remain within corrective range: $|\phi_P - \phi_H| < \pi/2$
- Feedback consistently stabilizes rather than destabilizes

At 2Hz and 16Hz (instability zones):

- Elevated lag CV (approaching 5.0, outliers >20)
- Increased alternans (78% and 92%)
- Pump-rhythm phase matching deteriorates
- Phase delays approach or exceed $\pi/2$, where sine function changes sign
- Risk of feedback inversion increases

This frequency dependence suggests the cardiac system evolved to operate at frequencies where **phase margin**—the safety buffer before feedback becomes destabilizing—is maximized. The 8Hz stability window in mice (corresponding to ~4-5 Hz or 60-75 bpm in humans) represents the sweet spot where pump cycling frequency naturally entrains to cardiac rhythm with minimal phase error accumulation.

Clinical interpretation: When ATP depletion, membrane disorder, or mitochondrial dysfunction increases pump-level phase drift, the phase delay widens from (for example) 20° to 60°. If this widening occurs while the system is forced outside its natural frequency band—either by tachycardia (excessive sympathetic drive) or by loss of frequency reserve (inability to modulate rate appropriately)—the phase delay can exceed 90° ($\pi/2$ radians), at which point the corrective feedback term inverts and becomes **destabilizing feedback**. The SA node then receives ionic currents that drive it away from rather than toward stable rhythm, manifesting as sustained alternans, beat-to-beat phase opposition, and ultimately spatial desynchronization that provides substrate for re-entrant arrhythmias.

Our elevated lag variability finding is thus not merely kinetic slowing—it represents **erosion of phase margin**, the narrowing of the temporal window within which feedback remains corrective rather than disruptive. In control theory terms, the system's phase margin:

$$PM = 180° - |\phi_P - \phi_H|$$

shrinks as pump-rhythm phase drift increases. When phase margin approaches zero, the system transitions from stable phase-locking to oscillatory instability—the cardiac manifestation being alternans and arrhythmia.

This interpretation reframes HRV loss and arrhythmia risk not as failure of the master pacemaker itself, but as **narrowing of the phase margin** that normally protects hierarchical cardiac synchronization. The heart retains its intrinsic firing capability (command authority preserved), but loses the phase precision in upward feedback required to maintain adaptive rhythm stability. In this framing, the upward coupling strength K_{up} in our feedback equation represents not just signal amplitude but **effective phase margin**—as K_{up} declines toward the critical threshold K_{up}^*, the temporal window for corrective feedback narrows until phase delays push the system into the destabilizing regime where $\sin(\phi_P - \phi_H)$ becomes negative. Therapeutic interventions should therefore target restoration of pump-rhythm phase coherence—reducing phase drift through metabolic support, membrane stabilization, and pump complex

integrity—to widen phase margin before the system crosses into the phase-cancellation regime where feedback becomes self-destructive.

Why This Framework Resolves Apparent Paradoxes

Paradox 1: Preserved E-C coupling with declining HRV

Traditional view: Cannot be reconciled—if coupling is intact, HRV should be normal.

Our framework: Layer 2 (E-C) remains coherent while Layer 1 (pumps) degrades and Layer 3 (HRV) fails due to upward propagation of accumulated phase noise. Single-beat coherence ≠ multi-beat variability.

Paradox 2: ATP depletion predicts arrhythmia independent of ejection fraction

Traditional view: Confusing—if contractile function is preserved (normal EF), why arrhythmia?

Our framework: ATP depletion degrades Layer 1 pump-rhythm coupling (K_{up} declines), leading to phase diffusion that manifests as arrhythmia via Layer 3 HRV collapse, even though Layer 2 E-C coupling and contractile function remain architecturally intact.

Paradox 3: Ex vivo tissue appears synchronized despite known in vivo HRV decline

Traditional view: Preparation artifact—ex vivo measurements don't reflect in vivo reality.

Our framework: Both are real. Ex vivo optical mapping (as performed by Ou et al.) captures Layer 2 (ms timescale, single beats) where synchrony is preserved. In vivo HRV captures Layer 3 (seconds-minutes timescale, integrated) where accumulated Layer 1 noise becomes visible. The paradox resolves through **timescale-dependent observation windows**.

Falsifiable Predictions and Experimental Roadmap

Our three-layer framework generates specific, testable predictions that distinguish it from alternative models:

Prediction 1: Pump-layer coherence will decline before Vm-Ca PLV

Test: Measure NKA α-subunit dimerization stability, lipid-raft integrity, or pump-phase synchronization (via FRET, voltage-sensitive dye targeting, or computational inference from ionic current kinetics) in parallel with Vm-Ca optical mapping.

Expected result: Pump coherence metrics will show Sham vs TAC differences and decline with age/stress, while Vm-Ca PLV remains preserved—confirming that Layer 1 desynchronization precedes Layer 2 disruption.

Discriminating power: If Vm-Ca PLV declines before or simultaneously with pump coherence, the framework is falsified and desynchronization originates within E-C machinery rather than upstream.

Prediction 2: Ex vivo lag CV correlates with in vivo HRV entropy

Test: Perform in vivo ECG recording to quantify HRV metrics (sample entropy, DFA α1, 0.1 Hz power) in conscious animals, then sacrifice for ex vivo optical mapping to measure lag CV. Analyze correlation within subjects.

Expected result: Animals with higher ex vivo lag CV will show lower in vivo HRV entropy, directly demonstrating that Layer 2 temporal variability predicts Layer 3 systemic desynchronization.

Discriminating power: If no correlation exists, Layer 2 lag CV does not propagate to Layer 3 HRV decline, invalidating the upward transmission mechanism.

Extension: Calculate transfer entropy $T_{\text{Vm→Ca}}$ and $T_{\text{Ca→Vm}}$ from dual optical mapping time-series data using established algorithms. Compare transfer entropy values to lag CV within subjects and across Sham vs TAC groups. Expected result: Transfer entropy will correlate inversely with lag CV (higher lag variance = lower information transfer), directly quantifying information loss in bits per beat.

Prediction 3: Pump-timing interventions reduce lag variability without altering PLV

Test: Apply interventions targeting Layer 1 pump function:

- Low-dose cardiac glycosides (NKA stabilization)
- SERCA activators (CDN1163, istaroxime)
- Membrane lipid-raft stabilizers (cholesterol modulation, sphingomyelin supplementation)
- Mitochondrial-targeted antioxidants (MitoQ, SS-31)

Measure both lag CV and PLV before/after intervention.

Expected result: Successful interventions will reduce lag CV (improved K_{up}) and decrease alternans incidence at frequency extremes (2Hz and 16Hz) without significantly changing PLV (Layer 2 already coherent), demonstrating that pump-layer targeting specifically addresses phase-margin erosion. Critically, effective interventions should shift the U-shaped alternans curve downward and widen the stability window, reflecting restored phase coherence across a broader frequency range.

Discriminating power: If interventions require simultaneous PLV improvement to reduce lag CV, E-C coupling is the primary dysfunction target rather than pump-rhythm interface.

Quantitative Bridge: From Lag CV to Critical Coupling Threshold

The reanalyzed data enable preliminary quantification of the relationship between observable metrics (lag CV, alternans incidence) and the theoretical coupling parameter K_{up}:

At 8Hz (optimal coupling):

- Lag CV ≈ 2.5 (median)
- Alternans incidence = 65%
- Interpretation: K_{up} substantially exceeds K_{up}^*, system operates in stable regime

At 2Hz and 16Hz (suboptimal coupling):

- Lag CV ≈ 5.0 (median, outliers >20)
- Alternans incidence = 78-92%
- Interpretation: K_{up} approaches K_{up}^*, system near stability boundary with frequent threshold crossings

Proposed model for critical threshold: $$K_{up}^* \approx \frac{1}{\text{lag CV}_{\text{critical}}} \times \text{scaling constant}$$

where lag CV$_{\text{critical}}$ ≈ 5-10 based on the transition from stable to alternans-dominated dynamics. This predicts that **lag CV >5 indicates impending loss of coupling stability**, providing a quantitative biomarker threshold.

Future calibration: Direct measurement of NKA or SERCA pump synchronization in parallel with lag CV will enable precise determination of the lag CV → K_{up} mapping function and validation of the critical threshold prediction.

Clinical and Therapeutic Implications

If HRV decline reflects pump-rhythm desynchronization (Layer 1) propagating through preserved E-C coupling (Layer 2) rather than primary contractile failure, therapeutic strategies should prioritize:

Primary targets (Layer 1 restoration):

1. **Metabolic efficiency:** Optimize ATP production and delivery (substrate selection, mitochondrial function, oxygen delivery)
2. **Membrane integrity:** Stabilize lipid-raft microdomains that organize pump complexes (phospholipid composition, cholesterol homeostasis)

3. **Pump complex stability:** Maintain NKA α-subunit dimerization and β-subunit interactions (glycoside binding site modulation, chaperone support)

Secondary targets (Layer 2 kinetic optimization): 4. **SERCA facilitation:** Enhance calcium reuptake kinetics without disrupting architectural coupling 5. **RyR2 stabilization:** Reduce oxidative modification and aberrant leak 6. **Action potential duration management:** Maintain appropriate frequency-dependent APD shortening

Avoid overemphasis on:

- Contractile protein modifications (Layer 2 architecture already preserved)
- Purely symptomatic HRV interventions (Layer 3 is downstream consequence)

Diagnostic Innovation: E-C Lag Variability as Functional Stress Test

E-C lag CV measured across frequency ranges could serve as an **early functional biomarker** for impaired pump-rhythm coupling reserve:

Proposed clinical protocol:

1. Measure lag CV at rest (4-5 Hz equivalent for humans, ~60-75 bpm)
2. Measure lag CV during controlled pacing or exercise (frequency stress test)
3. Quantify lag CV increase with frequency deviation from resting rate

Interpretation:

- Normal: Lag CV remains <3 across frequency range, U-shaped curve shallow
- Early dysfunction: Elevated lag CV at non-physiological frequencies, steep U-shape
- Advanced dysfunction: Elevated lag CV even at rest, alternans at physiological rates

Clinical advantage: This metric identifies patients at risk for HRV decline and arrhythmia **before structural remodeling becomes evident on standard echocardiography or before ejection fraction declines**. It assesses functional reserve at the pump-rhythm interface rather than downstream contractile capacity.

Limitations and Scope

Reanalysis of existing data: This work represents computational reanalysis of publicly available dual optical mapping data generously provided by Ou et al. (2021). We applied novel analytical approaches (phase-locking analysis, temporal lag quantification, hysteresis loop orientation) to extract insights not reported in the original publication. However, we did not perform the experiments, and our interpretations are constrained by the original experimental design:

- Isolated, Langendorff-perfused heart preparation normalizes many in vivo differences (controlled perfusion, constant temperature/oxygenation, absence of autonomic input)
- TAC model at 4 weeks represents acute pressure overload; chronic models may show different patterns
- Optical mapping resolution (250 Hz, 4ms) limits detection of sub-millisecond pump dynamics

Unmeasured components: The original dataset does not include:

- NKA or SERCA pump phase synchronization (Layer 1 coherence)
- In vivo HRV from the same animals (Layer 3 correlation)
- Mitochondrial membrane potential or ATP/ADP ratios (energetic state)
- Lipid-raft organization or pump complex dimerization (structural integrity)

The feedback-coherence collapse model is therefore a testable systems-level interpretation motivated by our reanalysis and supported by extensive literature, but requiring direct validation of the predicted Layer 1 → Layer 2 → Layer 3 causal chain through comprehensive cross-scale measurements.

Statistical considerations: Our mixed-effects model accounts for within-slice correlation but does not address potential regional heterogeneity within hearts or temporal drift across the recording session. Future studies should incorporate spatially-resolved analyses and longer recording durations to characterize spatial and temporal dynamics of coupling variability.

Connection to Broader Theoretical Framework

The pump-rhythm interface represents a **fundamental boundary in biological oscillator systems**: the point where continuous metabolic processes (pump cycling) must entrain to discrete rhythmic events (heartbeats). This boundary condition appears throughout biology:

- Neural spike timing vs continuous dendritic integration
- Circadian clock genes (continuous transcription) vs behavioral rhythms (discrete activity bouts)
- Cellular ATP production (continuous) vs cell division (discrete phases)

The cardiac system provides an experimentally tractable model for understanding how hierarchical oscillator networks maintain coherence across timescales and how desynchronization propagates when subordinate oscillators lose coupling strength. The principles revealed here—upward feedback dependence, phase-margin constraints, information channel degradation, and timescale-dependent observation of dysfunction—may represent general features of biological control systems that span molecular, cellular, organ, and organismal levels.

Conclusions

Reanalysis of dual voltage-calcium optical mapping data from Ou et al. (2021) reveals that cardiac excitation-contraction coupling maintains robust architectural synchronization—preserved phase-locking, normal temporal sequence, and constant coupling gain—across physiological pacing rates and in acute pressure-overload heart failure. The preservation of these architectural features **despite elevated temporal variability** establishes that desynchronization does not originate within contractile machinery itself (Layer 2), but rather at the upstream pump-rhythm interface (Layer 1) where continuous ionic and metabolic oscillators must entrain to pulsatile cardiac rhythm.

We propose a three-layer desynchronization cascade in which:

1. **Layer 1 (Microscopic):** Na^+/K^+-ATPase and SERCA pump complexes lose phase-biased synchronization to cardiac rhythm due to ATP limitation, membrane disruption, or mitochondrial dysfunction, generating μs-ms timing noise
2. **Layer 2 (Organ):** Excitation-contraction coupling remains architecturally coherent but transmits Layer 1 timing noise as elevated beat-to-beat lag variability (CV 2.5-5.0)
3. **Layer 3 (Systemic):** Accumulated phase errors propagate through autonomic feedback loops, manifesting as reduced HRV entropy and increased arrhythmia risk despite preserved contractile function

This framework resolves the apparent paradox of preserved tissue-level synchrony with declining systemic variability, explains why ATP depletion predicts arrhythmia independent of ejection fraction, and generates specific falsifiable predictions for cross-scale validation. The U-shaped frequency dependence of lag variability and alternans demonstrates that cardiac stability depends on operating near the natural resonance frequency where subordinate oscillator coupling (K_{up}) exceeds the critical threshold (K_{up}^*) and phase margin is maximized, suggesting evolutionary optimization of pacemaker frequency to minimize phase-cancellation risk while maximizing pump-rhythm coherence.

The master oscillator is enslaved to the coherence of its subordinate oscillators. When upward feedback quality degrades below the master's correction bandwidth, the phase margin protecting stable rhythm narrows until feedback timing delays approach the point where corrective signals invert to destabilizing signals. Systemic failure emerges not from loss of command authority, but from loss of the synchronized information flow and phase precision required for adaptive stability. Therapeutic strategies should therefore target Layer 1 pump-rhythm coupling—metabolic efficiency, membrane integrity, and pump complex stability—to restore information channel fidelity and widen phase margin, rather than focusing exclusively on downstream contractile modifications in an architectural system that remains fundamentally coherent.

SIGNIFICANCE STATEMENT

Reanalysis of publicly available dual optical mapping data reveals that the heart's intracellular excitation-contraction machinery remains architecturally synchronized even under pressure overload: phase coherence is preserved while kinetics degrade. This pattern localizes the earliest desynchronization to the ionic pump-rhythm interface (Layer 1) rather than within calcium-release machinery (Layer 2). The three-layer cascade model predicts that microscopic loss of pump entrainment propagates upward as heart rate variability reduction in vivo, establishing a mechanistic bridge from ion-pump timing dynamics (μs-ms) to whole-body autonomic variability (seconds-minutes). E-C lag variability serves as a measurable proxy for pump-rhythm coupling strength and phase margin erosion, providing an early functional biomarker for cardiac desynchronization before structural remodeling becomes evident. Reframing cardiac failure as information channel degradation with phase-cancellation vulnerability—where subordinate oscillators can no longer supply synchronized timing information within the corrective phase window—reveals that therapeutic targets should restore Layer 1 coherence and widen phase margin rather than Layer 2 contractile force. The heart functions as master oscillator whose phase stability fundamentally depends on coherent upward feedback; when that information flow degrades and phase delays exceed corrective range, systemic failure emerges despite preserved contractile architecture.

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DATA AVAILABILITY

This work represents reanalysis of publicly available data. The original dual voltage-calcium optical mapping recordings are available at:

Primary data source: Ou, X., Choi, B.-R., *et al.* (2021). Data descriptor: Dual voltage-calcium optical mapping dataset of ventricular fibrillation in Langendorff-perfused mouse hearts. *Scientific Data* 8, 260. https://doi.org/10.1038/s41597-021-01085-5

Figshare repositories:

- Sham C57 murine data: https://figshare.com/articles/dataset/11936610
- TAC C57 murine data: https://figshare.com/articles/dataset/11931666

All code for our reanalysis (phase-locking analysis, lag variability quantification, hysteresis loop orientation, alternans detection, and mixed-effects modeling) is available at [https://github.com/Entient/Synchronization-Theory].

COMPETING INTERESTS

The authors declare no competing interests.