Conclusions—Our study demonstrates that the interaction among \( V \), \([Ca]_i\) and \([Na]_i\) causes slow on–off switching (or intermittency) of APs with and without EADs have been widely reported and theoretical studies have related this on–off switch of AP duration in cardiac myocytes and EAD-mediated arrhythmias and suggests a novel possible mechanism for intermittency of cardiac arrhythmias. (Circ Arrhythm Electrophysiol. 2015;8:1472-1480. DOI: 10.1161/CIRCEP.115.003085.)

Key Words: action potentials • arrhythmias, cardiac • calcium • feedback • sodium

Cardiac arrhythmias, the leading cause of sudden cardiac death, often occur intermittently in patients. Both premature ventricular complexes and ventricular tachycardia are intermittent phenomena, occurring every few minutes to several days. As a major cause of cardiac arrhythmias, early afterdepolarization (EAD) can be induced by either spontaneous calcium \((Ca)\) releases and subsequent augmentation of the sodium \((Na)\)-Ca exchanger current \((I_{\text{NCX}})\) or by voltage \((V)\)-related instabilities because of reactivation of L-type Ca \((I_{\text{CaL}})\) or sodium \((I_{\text{Na}})\) currents. Regardless of its ionic mechanisms, EAD is sensitive to the net transmembrane current at its takeoff \( V \), whereby an inward or outward shift can turn EADs on or off. Spontaneous fluctuations between action potentials \((APs)\) with and without EADs have been widely observed experimentally and in simulations. Modeling and theoretical studies have related this on–off switch of EADs to the stochastic opening of \( I_{\text{CaL}} \) or the chaotic outcome of the interaction between the window \( I_{\text{CaL}} \) and K currents. However, prior work has focused primarily on mechanisms of EAD occurrence on a beat-to-beat basis, but not on how cellular EADs may occur intermittently and cause arrhythmias on a longer time scale. Furthermore, lack of consideration of the interplay among \( V \), \([Ca]_i\), and \([Na]_i\) in previous studies limited our understanding of EAD dynamics and their arrhythmia consequences.

The interaction between \( V \) and \([Ca]_i\) has proven important in the induction of complex \( V \) dynamics and subsequent arrhythmia. However, previous studies focused on conditions favoring Ca instability (such as alternans and oscillations), where cells were abnormally overloaded with Ca and \([Na]_i\) overload was considered relevant in that it contributes to \([Ca]_i\), loading. These limit our understanding of EADs.
WHAT IS KNOWN
- Early afterdepolarizations (EADs) are major triggers for cardiac arrhythmias which often occur intermittently in patients, from every few minutes to several days.
- Irregular fast-scale switches between action potentials (APs) with and without EADs at individual beats have been attributed to ion channel refractoriness, biological noise, or both, and suggested to be chaotic, which leads to cardiac arrhythmias via chaos synchronization.

WHAT THE STUDY ADDS
- We observed slow-scale on-off switches of AP trains with and without EADs in experiments and simulations, due to a bistable switch that emerges from interactions among intracellular [Ca], AP duration, and slow changes in intracellular [Na].
- Bistable switches of cellular EADs provided novel triggers and functional substrates for intermittent arrhythmias in homogeneous tissue, not previously appreciated.
- Biological noise can mask this slow-scale bistable switch, making it more difficult to discern experimentally.

Experiments
All animal procedures were approved by the University of California Davis animal welfare committee and conform to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health. Rabbit ventricular myocytes were isolated as previously described. Briefly, hearts were excised from adult male New Zealand rabbits and mounted on a Langendorff perfusion apparatus. Collagenase and protease were used to digest the hearts at 37°C, followed by mechanical dispersion to yield the single cells. Isolated myocytes were stored at room temperature for ≤8 hours before experiments. The myocytes were allowed to adhere to coverslips for 5 to 10 minutes before experiments. Current clamp recording was performed in cell-attached patch mode to minimize myocyte perturbation. Heat-polished glass pipettes were filled and cells bathed with a normal Tyrode’s solution containing (in mmol/L): 140 NaCl, 4 KCl, 1 MgCl₂, 1.8 CaCl₂, 5 HEPES free acid, 5 HEPES sodium salt, 11 glucose, pH 7.4 with NaOH at 37±1°C. AP recordings were performed with an Axopatch 200 amplifier (Molecular Devices) at a sampling rate of 25 kHz. APs were elicited every 2 or 3 s by injecting suprathreshold depolarizing currents (<2-fold the diastolic threshold of excitation). After APs reached steady state, partial block of the rapidly activating delayed rectifier K current (Iₖ) with E-4031 (100–200 nmol/L) was used to prolong AP repolarization and favor EAD generation. Data were analyzed using custom-made software written in MATLAB (The MathWorks Inc).

Results
Quasi-Periodic On–Off Switch of EADs in Experiments and Simulations
We induced EADs in rabbit ventricular myocytes by reducing Iₛₑₙ. Similar to previous studies, AP changed from beat to beat (Figure 1A, left) and by switching between 2 major states: short APs without EADs and long APs with 1 or 2 EADs (Figure 1A, gray versus pink box). The fast fluctuations between EAD and no EAD states (stars in Figure 1B) are expected. But the quasi-periodical on–off switch of EADs at a slow time scale is unprecedented, where the AP mostly maintained the same state for several beats before flipping to the other state (Figure 1A).

To understand the mechanisms underlying these previously unreported slow-scale transitions between APs with and without EADs, we performed simulations using a well-established

Methods
To understand the mechanism of experimentally observed intermittent EADs, we used a well-established rabbit ventricular myocyte model, which includes membrane ionic currents and [Ca] and [Na] dynamics (https://somapp.ucdmc.ucdavis.edu/Pharmacology/bers/). To enable EAD generation, we shifted the steady-state activation gate of Iₛₑₙ by −5 mV and increased the maximum Iₛₑₙ conductance by 60% (unless otherwise specified) to facilitate Iₛₑₙ reactivation. This approach has been previously implemented for EAD simulation in theoretical studies, and in part mimics alterations of Iₛₑₙ in response to β-adrenergic receptor activation or oxidative stress. We paced the cell model at a cycle length of 1.8 s to further prolong repolarization and favor EAD occurrence. Maximum pump rate of the Na–K ATPase (Vₛₑₙ) was increased by 20%. Simulations are performed in single cells, and 1-dimensional (1D, 4.5 cm) and 2D homogeneous tissues (4.5×4.5 cm). To speed up simulations, we assumed a rapid equilibrium approximation for 3 fast buffers (SLL, SLH, and CSQN). All model equations were implemented in C++ and solved with an adaptive time step (with the smallest integration time step being 1 μs at the AP upstroke). The electric diffusion coefficient is 0.001 cm²/ms; the space step is 0.015 cm, roughly the size of a myocyte.

In this study, we examined how interactions among V, [Ca] and [Na] affect the intermittent occurrence of EADs and the on–off switch that controls transitions. We performed experiments in rabbit ventricular myocytes exhibiting intermittent EADs, that is, repeating cycles of EADs appearing for numerous consecutive beats followed by trains of APs without EADs (a sort of bistable state). We then used dynamical systems analysis in a computational ventricular myocyte model to investigate the underlying mechanism. We found that a positive feedback between [Ca] and V, because of a dominance of inward Iₛₑₙ over Ca-dependent inactivation of Iₛₑₙ, causes bistability, that is, the coexistence of 2 stable states. Slow changes in [Na] cause transitions between a stable steady state at which EADs are seen, and a second stable state where EADs are suppressed. Simulations in 2-dimensional tissue also showed how this bistable switch of cellular EADs could contribute to cardiac arrhythmias at the tissue level.

in the absence of Ca instability, such as long-QT syndrome, where EADs may occur mainly because of V instability (eg, because of reactivation of Iₛₑₙ) favored by prolonged AP duration (APD). In these situations, EADs further prolong APs, which can increase [Ca] via prolonged opening of Iₛₑₙ and reduced diastolic time for Ca efflux. The [Ca] gain further prolongs AP via augmented inward Iₑₛₑₙ forming a positive APD-[Ca] feedback. These changes in [Ca] are also expected to affect [Na], which will in turn affect V stability via Na-dependent Iₛₑₙ and Na–K pump current (Iₛₑₙ). At higher [Na] shifts in both Iₛₑₙ and Iₛₑₙ can affect APD, but [Na] changes over a slower time scale than V and [Ca] (eg, minutes) in cardiac myocytes.
model of the rabbit ventricular AP and Ca transient. To favor EAD formation in the model, Ca current (I_{Ca,L}) conductance (G_{Ca}) was increased and the activation V-dependence was shifted –5 mV (similar to adrenergic modulation; Figure 2A, 2B, and 2D; Figure 1A in the Data Supplement). This resulted in EADs induced by I_{Ca,L} reactivation (Figure 1B; Figure II in the Data Supplement). Similar to our experiments (Figure 1B), the abrupt switches between short and long APD states occurred after many beats (Figure 2D top, shows one full period). APD switches between short and long states (Figure 2A, top) and the long APDs exhibit 1 or 2 EADs (defined as a net depolarization during the AP plateau; Figure 2B, top).

Mechanism of Slow-Scale EAD On–Off Switch

Simulations enabled us to investigate mechanisms related to [Ca], [Na], and AP dynamics. Diastolic and peak [Ca] and sarcoplasmic reticulum Ca load also exhibit switch-like periods similar to the APD (Figure 2A, 2B, and 2D; Figure IIIA in the Data Supplement). In contrast, [Na] accumulates (or dissipates) much more gradually (Figure 2A, bottom), reversing direction at the time of APD switch. Small increases in [Na] that occur with increasing heart rate are known to shorten APD because of [Na]-dependent outward shifts in both Na/Ca exchange and Na/K pump currents (I_{NCX} and I_{NaK}) during the delicate balance of AP plateau currents.\(^1\,2\) We hypothesize that the slow [Na] changes in Figure 2A might trigger the EAD on and off switches. That is, the progressive rise in [Na] during the long APD–EAD phase shifts I_{NCX} and especially I_{NaK} outwardly, favoring repolarization, which at a critical point abruptly shortens APD by preventing I_{Ca,L} reactivation. Conversely, the gradual dissipation of [Na] during the short APD and no EAD phase, shifts I_{NCX} and I_{NaK} inwardly.

Figure 1. Experimental quasi-periodic on-off switch of early afterdepolarizations (EADs). A, Action potential duration (APD) series (left) measured in a rabbit ventricular myocyte. Quasi-periodic slow transitions of APD between EAD (red shading) and no EAD states (gray shading) are apparent, where a, b, and c denote transitions between these 2 phases. Random jumps in APD also occur amid these phases. APs with EADs (black/red arrows) and without EADs (gray arrow) are shown. B, AP trains right straddling phase transition points. Only occasional beats (‘) interrupt the EAD (red bars) or no EAD phases (gray bars). Pacing cycle length=3 s.

Figure 2. Simulated quasi-periodic on-off switch of early afterdepolarizations (EADs). A, Simulated action potential duration (APD), diastolic intracellular calcium concentration ([Ca]), and sodium concentration ([Na]), showing quasi-periodic switches between short (without EADs, no. 1) and long APs (with EADs, no.2 and 3). Gray and red shadings indicate no EAD and EAD phases. B, Expanded AP, [Ca], and [Na] traces at nos. 1, 2, and 3 in A. Red lines indicate time of EAD takeoff, which precedes secondary Ca release (middle). C, Superimposed Na–K pump current (I_{NaK}; top), Na–Ca exchanger current (I_{NCX}; middle), and I_{NaK}+I_{NCX} (bottom) at the EAD switch off (red) and on (black) beat (ie, first and last beat of no EAD phase, arrows in D). D, AP, [Ca], and [Na], from A, showing the transition into intermittent EADs at time 0 (when G_{Ca} increases) in red- and gray-shaded regions.
Reducing repolarization, which abruptly prolongs APD by favoring $I_{\text{CaL}}$ reactivation (Figure IIA and IIB in the Data Supplement). Indeed, comparing the first and last beat of the no EAD phase (Figure 2C and 2D) confirmed that the net $[Na]_i$-dependent current ($I_{\text{NCX}}/I_{\text{NaK}}$) is slightly shifted outward during the switch-off AP plateau (Figure 2C, red versus black), which terminates EADs at the switch-off beat by suppressing the reactivation of $I_{\text{CaL}}$.

**Clamping $[Na]$ Abolishes EAD On–Off Switch and Unmasks Bistability**

How can such a small change in $[Na]$, and $[Na]$-dependent net current (Figure 2A and 2C) cause abrupt changes in APD (Figure 2A and 2D)? To understand this, and considering that $[Na]$ changes much more slowly than $V$ and $[Ca]$, we clamped $[Na]$ and reduced the $[Ca]_i$-$[Na]_i$-APD system to the $[Ca]_i$-$[Na]_i$-APD system. Clamping $[Na]$ eliminates oscillations in both APD and diastolic $[Ca]_i$ (Figure 3A–3C), but steady state APD and diastolic $[Ca]_i$ still depend on history. If $[Na]$ is clamped at 8.8 mmol/L during its decay (the no EAD phase), the APD remains short, and diastolic and peak $[Ca]_i$ stay low (Figure 3A–3C, black). But if the same $[Na]$-clamp (8.8 mmol/L) starts during the EAD phase, APDs remain long and $Ca$ transients large (Figure 3A–3C, red). Small perturbations can also cause a jump between these 2 steady states. For example, a small 1-ms inward current injection during the plateau of a short AP prolongs APD only transiently (Figure 3D black), but a 1.2% larger current can switch steady-state APs from short to long (with EAD; red). Thus, a sharp threshold current exists, which separates 2 stable AP states and forms bistability in the reduced system, when $[Na]$ is clamped.

Exploring the dependence of both APD and $[Ca]_i$ on $[Na]_i$ (Figure 3E and 3F), we found that bistability exists over a range of $[Na]_i$ ($\approx$8.65–8.9 mmol/L). Below this range, steady-state long AP with EADs is stable, whereas above it only short APs occur. Switches between the 2 quasi-stable states occur at different $[Na]_i$ (Figure 3E, vertical arrows), and short versus long APD have opposite effects on slow $[Na]_i$ changes (horizontal arrows), creating a hysteretic loop. Bistability and hysteresis are also seen in diastolic $[Ca]_i$ (Figure 3F), peak $[Ca]_i$, and sarcoplasmic reticulum $Ca$ load (Figure IIB in the Data Supplement).

To confirm the existence of bistable APs in the reduced $[Ca]_i$-APD system ($[Na]_i$ fixed at 8.8 mmol/L), Figure 4A shows an APD map, plotting $APD_\text{n}$ as a function of the prior $APD_\text{p}$ (see Data Supplement). Two discontinuous branches are seen (red), with each intersecting the black identity line ($APD_\text{n}=APD_\text{p}$) to reveal 2 stable fixed points (green dots) of the system (slope<1).

**Positive $[Ca]_i$-APD Feedback Underlies Bistability**

We next investigated the mechanisms of bistability in the $[Ca]_i$-APD system (Figure 5A; Figure IV in the Data Supplement). $[Ca]_i$ can affect membrane $V$ bidirectionally. On one hand, high $[Ca]_i$ elevates $V$ by increasing the inward $I_{\text{NCX}}$ and thus prolonging APD; on the other hand, high $[Ca]_i$ reduces $V$ by enhancing Ca-dependent inactivation of $I_{\text{CaL}}$, thus shortening APD. A prolonged APD, in turn, increases $[Ca]_i$ by increasing the Ca influx through $I_{\text{CaL}}$, and limiting diastolic time. Therefore, the $[Ca]_i$-APD subsystem can have positive or negative feedback between $[Ca]_i$ and APD (Figure IVB in the Data Supplement). Although in rabbit heart positive feedback via $I_{\text{NCX}}$ is typically dominant over negative feedback via $I_{\text{CaL}}$, the model allows manipulation either way. We promoted $I_{\text{CaL}}$ dominance on $V$ by decreasing $G_{\text{NCX}}$. To avoid $Ca$ overload-induced instabilities, we only decreased the electric conductance ($G_{\text{NCX}}$), but allowed normal Ca extrusion flux ($I_{\text{NCX}}$) as if NCX were electroneutral. Decreasing $G_{\text{NCX}}$ causes the bistable regime to shrink and disappear (Figure 4B), suggesting that strong $I_{\text{NCX}}$ is required for bistability. At low $G_{\text{NCX}}$, EADs can still occur even in the absence of bistability because of $I_{\text{CaL}}$ reaction (leftmost curve in Figure 4B). This implies that a large $I_{\text{NCX}}$ (dominant over $I_{\text{CaL}}$, Ca-dependent inactivation) is required for establishing a positive $[Ca]_i$-APD feedback (and hence bistability) but is not required for EAD formation. To further test this, when removing the positive feedback by setting $G_{\text{NCX}}=0$, we could not induce bistability, even when we additionally reduced K currents to favor EADs (Figure 4C; Figure V in the Data Supplement). We conclude that $I_{\text{NCX}}$ is critical for bistability because of positive $[Ca]_i$-APD feedback (Figure 5A; Figure IVB in the Data Supplement), not simply by causing AP prolongation and EADs.

**Slow Accumulation and Dissipation of $[Na]_i$ Constitute a Slow-Scale EAD Bistable Switch**

In the system with $[Na]_i$ clamped, a rapid increase in $[Ca]_i$ causes $[Na]_i$ accumulation through NCX on a much slower scale. This increased $[Na]_i$ in turn outwardly shifts $I_{\text{NCX}}$ and $I_{\text{CaL}}$ and limits diastolic $[Ca]_i$. The phase at the time $[Na]_i$ unclamped, a rapid increase in $[Ca]_i$; see Data Supplement). Stabilizing $[Ca]_i$ reverses the feedback, preventing bistability (Figure IVB in the Data Supplement).

**Figure 3.** Intracellular sodium concentration ($[Na]_i$) clamping abolishes the early afterdepolarization (EAD) on-off switch and unmasks bistability. A, Clamping $[Na]_i$ (at 8.8 mmol/L) during either no EAD (black) or EAD phase (red) eliminates oscillations in both APD (B) and intracellular calcium concentration ($[Ca]_i$; C). The phase at the time $[Na]_i$ clamps dictates the new stable steady-state APD and $[Ca]_i$. D, A suprathreshold perturbation (inward current, $-7.7 A/F$ for 1 ms, during the AP plateau of 5th beat, arrow) succeeds (red), whereas a 1.2% smaller perturbation fails (black) to switch APD from short to long steady state. E, Long (with EAD) and short APs (without EAD) coexist over a range of $[Na]_i$. Switches between long and short APDs (arrows) occur at different $[Na]_i$, revealing hysteresis. F, Bistability and hysteresis in diastolic $[Ca]_i$.
the slow [Na] dynamics and the fast [Ca]-APD subsystem. In phase plots of APD and [Na], the fast [Ca]-APD subsystem shows bistable APD (APD nullclines as black circles in Figure 5B). The slow [Na] variable increases linearly with APD ([Na] nullcline, Figure 5B black line). The intersection of these 2 nullclines, which identifies the system’s fixed point, does not occur in either stable APD nullcline with intermittent EADs, but crosses the unstable branch (Figure 5B). Thus, the APD oscillates around the bistable regime and forms a hysteresis loop (Figure 5B, red curves), corresponding to the quasi-periodic APD fluctuations at the steady state (Figure 2). This quasi-periodical oscillation in APD can also be considered a relaxation oscillator, formed by fast positive feedback-induced bistability and a slow negative feedback in dynamical systems.

These phase plots also illustrate how intermittent EADs form or are suppressed. For example, when $G_{\text{CaL}}$ is lower, the bistable APD regime shifts to lower [Na] (Figure 5C, blue circles). With smaller $I_{\text{Cal}}$, there is also decreased [Ca] and [Na], which shifts the [Na] nullcline (Figure 5C, blue line). In this lower $I_{\text{Cal}}$ scenario, the fixed point of the system anchors on the lower stable branch (Figure 5C, red star), forming a steady short AP without EADs (time <0 s in Figure 1). But an abrupt increase of $G_{\text{ncx}}$ can prolong APD and generate EADs quickly, shifting to the bistable regime from Figure 5B (red line and arrows in Figure 5C).

### Noise Can Mask the Slow-Scale EAD Bistable Switch

Previous experimental studies of EADs have not identified the intermittency described here. One potential explanation is that noise in the real biological system might mask the bistable switches of EADs (Figure 1A, black). In addition, many cardiac ion channels exhibit refractoriness, which can lead to irregular APD, especially at fast rates. Both phenomena may limit the observability of bistable switches and intermittent EADs experimentally.

To examine how biological noise may alter bistability detection, we used Poincaré plots (ie, APD of the current beat [APD$_n$] versus previous beat APD [APD$_{n-1}$]). Bistability, as in the model here, leads to 2 separate clusters (with or without EAD) along the identity line (Figure 6Aa, red and black circle, respectively). However, if the switch is because of refractoriness, an EAD at APD$_{n-1}$ would always lead to a shorter APD$_n$ and vice versa (giving no points in the red circle region). Therefore, the clusters in black and red circles along the identity line in these Poincaré plots are consistent with an underlying bistable switch of EADs. Biological noise, because of variance of individual currents during the sensitive plateau phase, will tend to scatter points without specific patterns. This is shown in Figure 6A, with noise of different amplitudes ($\sigma$) applied to membrane V in the model with bistability. Without noise, >80% of the total beats are along the identity line (Figure 6Aa and 6Ab, left). As the noise level increases, the variability within each cluster increases and more points occur in the top-left and bottom-right quadrants, indicative of individual beats that jump between EAD and no EAD (Figure 6Aa and 6Ab, left to right). Even then, the red EAD cluster is discernable, even under conditions where periodicity is hard to detect in an AP series because of high noise (Figure 6Ac, blue). We could not detect coexistence of EAD versus no EAD clusters in the model without bistability ($G_{\text{ncx}}=0$), especially with noise (Figure VI in the Data Supplement).

### Figure 4
A dominant inward Na–Ca exchanger current ($I_{\text{NCX}}$) over L-type Ca current ($I_{\text{CaL}}$) Ca-dependent inactivation (CDI) imposes a positive intracellular calcium concentration ([Ca]$_i$)–action potential duration (APD) feedback and underlies bistability. A, APD map shows 2 discontinuous branches (red). Intersection of identity line (APD$_n$=APD$_{n-1}$) denotes 2 stable states (green dots). B, Attenuating the positive feedback between APD and [Ca]$_i$ by decreasing $G_{\text{NCX}}$ (not $J_{\text{ncx}}$) progressively suppresses bistability. C, Early afterdepolarization (EAD) alone (without positive [Ca]$_i$-APD feedback by setting $G_{\text{ncx}}$ to 0) fails to produce bistability in either periodic 1 EAD (cyan) or irregular EADs (pink). For details, see Figure VB and VC in the Data Supplement.

### Figure 5
A slow intracellular sodium concentration ([Na]) accumulation and depletion constitute an early afterdepolarization (EAD) bistable switch. A, Schematic diagram of the interaction between [Na]$_i$ and [Ca]$_i$-APD. Solid indicates activation; and dashed, suppression. B, APD-[Na]$_i$ phase plot shows hysteresis loop because of bistability. APD nullcline (circles) and [Na]$_i$ nullcline (black line, calculated by AP clamp in Figure IB in the Data Supplement) do not intersect either stable branch, so APs of the whole system oscillate around the bistable regime (red and arrows). C, Decreasing $G_{\text{ncx}}$ shifts both nullclines to lower [Na]$_i$ (blue), allowing intersection at the low stable branch (red star). Thus, the system stays at a steady-state short AP without EADs (corresponding to traces at t<0 ms in Figure 2). Red lines and arrows illustrate the APD sequence in Figure 2. APD indicates action potential duration.
We applied the APD Poincaré analysis to our experimental recordings: Figure 6B shows results from 2 cells that clearly demonstrate EAD and no EAD clusters (Figure 6Ba is the AP trace in Figure 1, and Figure 6Bb is the AP trace in Figure VII B and VIIC in the Data Supplement). In contrast, 2 other myocytes did not exhibit the same Poincaré pattern (Figure VIII in the Data Supplement). We infer that in some cases (half of our small sample) bistability may not be the major mechanism of intermittent EADs. However, since noise obscures even the Poincaré pattern, the negative cases may be equivocal (e.g., cell 4 versus 3 in Figure VIII in the Data Supplement).

**Bistable AP Switch Induces Intermittent Cardiac Arrhythmias**

How do bistable cellular APs contribute to cardiac arrhythmias at the tissue level? The slow \([Na]i\) accumulation/dissipation around its bistable regime forms a hysteresis bistable switch (Figures 1, 2, and 5). One can imagine that cells in different regions of cardiac tissue may be out of phase at either EAD state or no EAD state, depending on their history or initial conditions. Therefore, bistability and hysteresis might (even in homogenous tissue) underlie repolarization heterogeneity, which can cause both conduction block for reentrant arrhythmias and ectopic beats for focal arrhythmias, as demonstrated in our 1D and 2D tissue simulations (Figure 7).

In a homogenous 1D cable, we set different initial conditions (in \([Na]i\)) for one half of the cable versus the other half (Figure 7A bottom-left, red versus blue). Before gap-junctional coupling, APs are out of phase in the cable (one end with EADs, the other without; Figure 7A top, AP traces a0 versus b0), causing repolarization heterogeneity. When cells are electrically coupled, this heterogeneity facilitates EAD propagation (from sites with EADs to those without EADs) and formation of both ectopic beats (Figure 7A middle, AP traces a1 and b1) and conduction block (manifested by the space–time plot, Figure 7A bottom). EADs occur intermittently in all cells, but can propagate bidirectionally as they do in homogeneous tissue (arrows, Figure 7A). Heterogeneous initial conditions in variables other than \([Na]i\) can also lead to repolarization heterogeneity and PVCs, as long as they set cells at different phases (EAD versus no EAD, not shown).

In a homogeneous 2D tissue, we set different initial conditions for the left-bottom quarter versus the remaining portion of tissue (Figure 7B bottom-left, red versus blue). Periodic pacing was applied at the left-bottom corner. We recorded the pseudo-ECG (Figure 7B top) and 3 voltage traces along wave propagation (Figure 7B middle, AP traces 1, 2, and 3). EADs propagate and form ectopic beats as shown in the AP recordings (Figure 7B middle, arrows). To exemplify the spatiotemporal dynamics, we show voltage snapshots for part of the arrhythmias (blue bar; Figure 7B, bottom panels). EAD propagates from the EAD area other than the pacing site (snapshots at t=480, 516 ms) and forms ectopic beat (t=650 ms). As the firing rate of focal sites is much faster than the pacing rate, it interacts with the wave back of APs initiating from the pacing site, resulting in conduction block and reentry (snapshots at t=1206, 1610 ms). These interactions generate complex spatiotemporal patterns with highly irregular electric activity (snapshots at t=2814 ms) before self-terminating.

Interestingly, arrhythmias caused by bistability occur intermittently in both 1D cable (Figure 7A middle, AP traces) and 2D tissue (Figure 7B top, pseudo-ECG). Because EADs occur quasiperiodically as a result of bistability and hysteresis, the long-and-short APD pattern occur out of phase in space intermittently (Figure 7A, top). Therefore, the repolarization heterogeneity and EAD propagation spontaneously occur and terminate repetitively, giving rise to intermittent arrhythmias that are often observed in both clinics and experiments. Complex tissue dynamics of course would make observed intermittent arrhythmias like this difficult to connect mechanistically to the fundamental myocyte bistability discussed here. But that is indeed the underlying basis of the arrhythmic pattern in this simulation.
Discussion
In this study, we used a computational model and discovered how slow fluctuations in [Na], can cause intermittency in cardiac AP dynamics, with APD fluctuating between normal (no EAD) and abnormal (EADs). The main findings are (1) bistable APs (with and without EADs) can arise when [Na] is fixed and require positive [Ca]i-APD feedback; (2) this bistability leads to intermittent EADs, whereby APs switch quasiperiodically between long APs with EADs and short APs without EADs as [Na] slowly accumulates or dissipates around its bistable regime; experimental AP recordings showed intermittent EADs similar to those shown in simulations; (3) biological noise can mask underlying bistability, making it more difficult to discern experimentally; (4) intermittent EADs provide both an arrhythmia trigger and a substrate (causing repolarization heterogeneity) in homogeneous tissue and induce both reentrant and focal arrhythmias intermittently in cardiac tissue. We propose bistability as a novel mechanism for the switch on and off of EADs and for EAD-induced arrhythmias and offer a plausible mechanistic explanation for some of the intermittent cardiac arrhythmias widely observed both clinically and experimentally.1,2,30

Instability Arises From the Interaction Among Ca and Na Homeostasis and EADs
The cardiac AP is shaped by the balance between inward and outward currents, which interact with varying [Ca]i and [Na], during the cardiac cycle. Fluxes of both Ca and Na are critical for stable AP and efficient contraction.31 Instabilities in Ca cycling, such as Ca alternans, oscillations, and waves, can lead to membrane potential instabilities and cause arrhythmias.12–15 Na instabilities are mainly seen when [Na]i is elevated (as in heart failure19), which increase Ca loading and thus Ca-mediated membrane potential instability.31 However, large (≈ mmol/L) changes in global [Na]i are unlikely to occur in a short time,18,32 and the contribution of [Na]i to arrhythmias has been overlooked because of its slower dynamics (minutes33) compared with rapid changes in V and [Ca]. Here, we found that interaction between a stable Ca cycling and EADs is capable of inducing bistability (Figures 3 and 4), and the slow [Na]i dynamics (even within the physiological range) can cause the abrupt APD changes (Figures 2 and 5) and arrhythmias (Figure 7). Note that not only does the turn on and turn off of EADs alter [Ca]i and [Na]i but also changes in [Ca]i or [Na]i in turn regulate the switching on and off of EADs.

Mechanisms Underlying EAD On–Off Switch
Irregular beat-to-beat transitions between EAD and no EAD beats have been studied in previous experiments7,8 and simulations6,9 and attributed to refractoriness, biological noise, or both.5,8,9 Previous studies showed that [Ca]i accumulation during the EAD period causes elevation of [Na]i, which then terminates the EADs.32 In our experiments, we observed both of these fast-scale and slow-scale switches (Figure 1), and our

Figure 7. Heterogeneity, ectopic beats, and intermittent arrhythmias in homogeneous tissue because of bistable switches. A, Intermittent early afterdepolarization (EAD) propagation in 1-dimensional (1D) cable. Action potentials (APs) from top (a0, a1) and bottom (b0, b1) of the cable are shown. Traces a0 and b0 are for uncoupled cells, showing out-of-phase bistable switches because of different initial conditions. When cells are electrically coupled (a1 and b1), EADs propagate intermittently. Space–time plot (bottom) shows one arrhythmic episode. Arrows denote EAD propagation. B, AP duration (APD) heterogeneities and ectopic beats form in 2-dimensional (2D) tissue because of intermittent EAD propagation. Top, Pseudo-ECG shows intermittent arrhythmias. Middle, AP trains along the AP propagation during 1 arrhythmia episode (blue bar below ECG). Bottom, Voltage snapshots show complex spatiotemporal patterns. [Na]i was initially set 0.3 mmol/L higher in the red areas for both 1D cable and 2D tissue.
simulations and analysis demonstrate that a bistable switch underlies the latter. Using APD Poincaré plots, we suggest that a bistable switch on and off of EADs may be common and exist even in the presence of apparently irregular APD sequences (Figure 6Bb; Figure VIIB in the Data Supplement) and be masked by noise, which makes the bistable states less detectable when only inspecting AP or APD traces (such as that in Figure VII B and VIIB, right in the Data Supplement).

We acknowledge that although bistability is consistent with the presence of these 2 clusters in the APD Poincaré plots, other cardiac memory effects may also contribute to the switch on and off of EADs and allow the coexistence of 2 clusters with and without EADs. Noise might also account for differences between model and experimental EAD waveforms.

In our model, the EAD on and off switch occurred and was analyzed with stable intracellular Ca cycling. We did not measure [Ca] during experiments, but our conditions were likely to prevent spontaneous Ca release during our AP recordings. That is, we expect low sarcoplasmic reticulum load in rabbit myocytes paced at slow rates without adrenergic activation. Spontaneous Ca release, which may occur with Ca overload, would complicate interpretation of the mechanisms here and warrants further investigation.

**Model Independence of Our Findings**

To what extent do our findings depend on the detailed ionic formulations and parameter values of the computational model used? To answer this question, we consider the conditions required for bistability and hysteresis.

A positive Ca-to-V coupling is required to establish a positive feedback between [Ca] and APD (Figure 4B). In this condition, the activation of the inward \( I_{\text{NCX}} \) by [Ca] is dominant over Ca-dependent inactivation of \( I_{\text{CaL}} \), so that a larger Ca transient leads to a longer APD, as seen in a variety of experimental conditions. Certain pathological conditions, where \( I_{\text{NCX}} \) and [Na] are up-regulated, such as heart failure, might favor bistability by strengthening the positive feedback between [Ca] and APD (in the presence of EADs; Figure 4C and 4D).

Various types of EADs have been explained by different ionic mechanisms (reviewed by Weiss et al), including \( I_{\text{CaL}} \) reactivation, enhanced Ca release and subsequent NCX augmentation and reactivating Na current. Even when initiated by the same ionic mechanism, EAD waveform and rising rate can vary depending on takeoff time and voltage. We argue that independent of the ionic mechanism or waveforms, all that is needed to obtain the nonlinear APD maps (Figure 4A) necessary for bistability is sensitivity of the EAD to changes in the net transmembrane current at its takeoff, allowing on-and-off behavior. This tends to occur when EADs are generated by large regenerative inward currents.

As discussed above, the slow dynamics of [Na] and its negative regulation of APD allow oscillations around the bistable regime for intermittent EADs (Figure 5). Alterations of either Ca- or Na-related currents often coexist with EADs in a variety of pathological conditions. These can also alter [Na] and push it to its bistable regime and form intermittent EADs.

Altogether, we expect our findings to hold true in any detailed ionic model that satisfies the above conditions and to not be parameter-specific. However, the specific [Na], [Ca], and APD range in which this bistability occurs would vary with model details, and the above conditions could well be fulfilled by different ionic mechanisms than we have highlighted.

**EADs-Mediated Arrhythmias and Bistability**

Cardiac arrhythmias are initiated when a triggering event encounters a substrate, that is, an electrophysiologically vulnerable region of tissue. For example, EADs were thought not to propagate in homogenous tissue, unless EADs are chaotic and form dynamical repolarization heterogeneity through chaos desynchronization. Here, we found that bistable APs with or without EAD can be induced because of the positive V-[Ca] feedback. This bistability amplifies small differences in initial conditions and induces large repolarization heterogeneity by setting cells to different phases without requiring chaotic EADs (Figure 7). Thus, besides chaos desynchronization, bistability provides another dynamical mechanism for EAD-mediated arrhythmias in homogeneous tissue. The bistable switch between APs with and without EADs provides an arrhythmogenic trigger and can generate large repolarization heterogeneity (substrate) in homogeneous tissue (Figure 7).

Cardiac arrhythmias are usually transient and intermittent in both experiments and clinics. For example, atrial fibrillation is shown to spontaneously self-terminate and reoccur before becoming chronic (ie, persistent). Premature ventricular complexes and ventricular tachycardia, which often precede ventricular fibrillation and sudden cardiac death, also occur intermittently. Cellular EADs found in most studies, especially in simulations, are persistent or transient in nature rather than intermittent, which causes a gap in our understanding of the cellular basis of intermittency of arrhythmias.

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**Disclosures**

None.

**References**


Slow [Na]i Changes and Positive Feedback Between Membrane Potential and [Ca]i Underlie Intermittent Early Afterdepolarizations and Arrhythmias
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SUPPLEMENTAL METERIAL

Calculation of the action potential duration (APD) map
In the reduced calcium ([Ca]i) -APD system, where intracellular sodium ([Na]i) is clamped (Fig. 4B), we constructed APD maps to illustrate the nonlinearity in the system for bistability. Denote the APD of the ith AP during periodic pacing by APDi and its associated diastolic interval (DI) by DIi. The APD map, i.e. the one-dimensional finite difference equation

\[ APD_n = f(APD_{n-1}) \]  

can be constructed from the APD restitution (or APD recovery curve), as shown by Yeti et al (1). The APD restitution assumes that at arbitrary pacing cycle length (PCL), APD is determined only by its preceding DI (Eq. 1),

\[ APD_n = g(DI_{n-1}) \]  \hspace{1cm} (Eq. 1)

By definition, \( PCL = APD + DI \), thus,

\[ APD_{n-1} = PCL - DI_{n-1} \]  \hspace{1cm} (Eq. 2)

Substituting Eq. 2 into Eq. 1, one has

\[ APD_n = g(PCL - APD_{n-1}) = f(APD_{n-1}) \]  \hspace{1cm} (Eq. 3)

To obtain the steady-state APD restitution, we paced the cell at an arbitrary DI_{n-1} till APD_n reaches its steady state. Then by varying DI_{n-1}, we determined the steady-state APD restitution (g). At a specific PCL (1.8 s in this study), Eq. 3 gives the APD map (f).

Since V is coupled to [Ca], (Fig. 3B), a more accurate map of the system would require [Ca] also. Although a different [Ca] level shifted the APD map, it did not change the solution qualitatively (Fig. S3A).
**Fig. S1. Dependence of APD on G_{Ca}.** A. With a -5 mV shift of the steady-state activation (SSA) gate of I_{Ca,L}, increasing G_{Ca} (> 1.57 folds) causes intermittent EADs within a range (red bar), after which EADs persist. B. Without the shift in I_{Ca,L} activation, increasing G_{Ca} alone prolongs APD but does not induce EAD (inset). To avoid spontaneous Ca release at long AP, EC₅₀ of the SR Ca release was increased by 50%.

**Fig. S2. EAD is due to I_{cal} reactivation.** Voltage (A), I_{Ca,L}(B), SR Ca (C) and I_{Na} (D) are aligned to show that I_{cal} reactivation precedes EAD take off and the secondary Ca release (dashed line). There is no I_{Na} reactivation underlying the EAD (D).
**Fig. S3. Hysteresis and bistability in peak [Ca] \(_i\) and SR Ca load.** **A.** Both peak [Ca] \(_i\) (top) and SR Ca load (bottom) corresponding to the APDs in Fig. 2 show similar oscillation to the diastolic [Ca] \(_i\) (Fig. 2A middle). **B.** Bistability exists in both peak [Ca] \(_i\) (left) and SR Ca load (right).

**Fig. S4. Schematic of the bi-directional coupling between [Ca] \(_i\) and membrane V in cardiac myocytes.** **A.** [Ca] \(_i\) regulates membrane V and thus APD through \(I_{\text{CaL}}\) and \(I_{\text{NCX}}\); APD in turn modulates [Ca] \(_i\) via Ca fluxes, \(J_{\text{Ca}}\). **B.** Simplified [Ca] \(_i\)-APD feedback schematic from A. Red solid – activation; blue dashed – suppression.
Bistability does not occur in the model with negative [Ca]-APD feedback

To further test the hypothesis that a strong positive [Ca]-APD feedback is required for bistable early afterdepolarization (EAD)-no EAD pattern, we investigated whether bistability can occur in EAD enabling models with negative [Ca]-APD feedback. We set the maximum conductance of the Na-Ca exchanger current ($G_{NCX}$) to 0 to ensure a negative [Ca]-APD feedback (Fig. 4Bb), while permitting Ca extrusion via $I_{NCX}$ ($J_{NCX}$). In this condition (reduced inward current) we additionally decreased potassium (K) currents (see figure legends) to achieve AP prolongation, $I_{CaL}$ reactivation and EAD formation (Fig. S3B) as in control (Fig. S1A). We investigated two cases: 1) periodic 1 EAD (Fig. S5B cyan, AP #2), and 2) irregular EADs (Figs. S5B pink (AP #3) and C). In both cases, APD decreases with increasing [Na], showing no bistability (Fig. S5D cyan and pink correspondingly). APD maps of the periodic 1 EAD reveal only one branch (the EAD branch) that forms one stable fixed point (Fig. S5D cyan). In the irregular EADs, nonlinearity exists and the APD map shows two branches (Fig. S5D pink). However, only one intersection is possible (Fig. S5D pink), as APD n negatively correlates to APD n-1 due to the negative [Ca]-APD feedback. This confirms that bistability requires a positive [Ca]-APD feedback, which occurs when $I_{NCX}$ dominates over $I_{CaL}$.

Fig. S5. Weakening the [Ca]-APD positive feedback abolishes bistability. A. APD maps are shifted when [Ca] is varied (green, 0.196 μM; red, 0.183 μM; and blue, 0.187 μM), but do not change qualitatively. B. Dependence of APD on $G_{Ca}$ when $G_{NCX}=0$ (both $G_{Kr}$ and $G_{to,s}$ are decreased to 5% in this analysis). As $G_{Ca}$ value increases to 1.4-fold, APD changes smoothly from no EAD (#1 black) to periodic 1 (low-amplitude) EAD occurs (#2 cyan, inset). Irregular EADs occur for larger $G_{Ca}$ (#3, pink). C. APD trace (top) and AP profiles of the first 4 beats (bottom) from #3 in panel B. D. APD maps for periodic 1 EAD and irregular EADs (seen in panel B and Fig. 4D). The black dotted line is the identity line. Only one intersection (green dot) is possible in both.
**Fig. S6. APD Poincaré plots in the model without bistable EAD switches \(G_{NCX}=0\).** A. Only one cluster (the no EAD cluster) exists, especially with noise. B. APD time series at the low and high levels of noise (\(\sigma=0.005 \text{ mV} \) (black) vs. \(\sigma=0.1 \text{ mV} \) (blue)). C. Fraction of total beats in EAD (red) and no EAD cluster (black) for various noise levels.

**Fig. S7. Coexistence of EAD and no EAD clusters in experiments.** A. APD Poincaré plot of Fig. 6Ba is redrawn to include the full axis range. Dotted line is the identity line. Red and black circles indicate the clusters with and without EADs respectively. B. APD Poincaré plot from the cell in Fig. 6Bb is redrawn to include the full axis range (left), along with the corresponding APD sequence (right). C. A sample of AP trace from the experiment in panel B.
Fig. S8. Recordings from two cells that do not show clear coexistence of EAD and no EAD clusters. A. APD sequences. B. APD Poincaré plots.

References