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TOPICAL REVIEW

Neuronal sodium channels: emerging components of the nano-machinery of cardiac calcium cycling

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Abstract Excitation–contraction coupling is the bridge between cardiac electrical activation and mechanical contraction. It is driven by the influx of Ca^{2+} across the sarcolemma triggering Ca^{2+} release from the sarcoplasmic reticulum (SR) – a process termed Ca^{2+}-induced Ca^{2+} release (CICR) – followed by re-sequestration of Ca^{2+} into the SR. The Na^+/Ca^{2+} exchanger inextricably couples the cycling of Ca^{2+} and Na^+ in cardiac myocytes. Thus, influx of Na^+ via voltage-gated Na^+ channels (Na_V) has emerged as an important regulator of CICR both in health and in disease. Recent insights into the subcellular distribution of cardiac and neuronal Na_V isoforms and their ultrastructural milieu have important implications for the roles of these channels in mediating Ca^{2+}-driven arrhythmias. This review will discuss functional insights into the role of neuronal Na_V isoforms vis-à-vis cardiac Na_Vs in triggering such arrhythmias and their potential as therapeutic targets in the context of the aforementioned structural observations.

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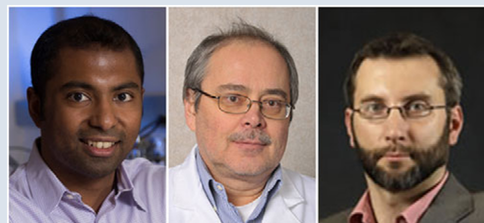
Abstract figure legend Top: Schematic diagram showing the protein machinery of cardiac Na^+–Ca^{2+} cycling showing a t-tubule and associated junctional SR. Microfolds in t-tubule are depicted based on recent findings. (Hong *et al.* 2014; Lavorato *et al.* 2015) Note the close proximity of neuronal sodium channels (nNaVs) to ryanodine receptor channels (RyRs) and the Na^+–Ca^{2+} exchanger (NCX). Differential shading of the interstitial space within the t-tubule and the cytoplasm within the dyadic cleft indicates local differences in ionic concentrations, particularly with respect to the bulk interstitium and cytosol respectively. Bottom left: During β-adrenergic stimulation in healthy hearts, Na^+ influx is enhanced secondary to CaMKII-mediated phosphorylation of nNaVs. This augments Ca^{2+} influx via reverse mode NCX, and in turn, to enhanced SR Ca^{2+} release via RyRs. Inset shows normal electrocardiogram resulting from normal Na^+–Ca^{2+} cycling. Bottom right: In diseased hearts, pathologically elevated Na^+ influx via nNaVs results in a larger Ca^{2+} influx via reverse mode NCX. This, particularly in the presence of elevated diastolic Ca^{2+} levels or RyR leak, can trigger arrhythmogenic diastolic Ca^{2+} release. Inset electrocardiogram shows premature beats and arrhythmias triggered by diastolic Ca^{2+} releases.

Abbreviations BIN1, bridging integrator 1; CaMKII, Ca^{2+}/calmodulin-dependent protein kinase II; CICR, Ca^{2+}-induced Ca^{2+} release; cNa_V, cardiac sodium channel; CPVT, catecholaminergic polymorphic ventricular tachycardia; ECC, excitation-contraction coupling; HF, heart failure; LCC, L-type Ca^{2+} channel; Na_V, voltage-gated Na^+ channel; NCX, sodium–calcium exchanger; nNa_V, neuronal sodium channel; RyR, ryanodine receptor; SR, sarcoplasmic reticulum; t-tubule, transverse tubule; TTX, tetrodotoxin; WT, wild-type.



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Cardiac calcium (Ca^{2+}) cycling involves the release of Ca^{2+} from intracellular stores, i.e. the sarcoplasmic reticulum (SR), prompted by Ca^{2+} influx across the sarcolemma – a process termed Ca^{2+}-induced Ca^{2+} release (CICR), and its re-sequestration into the SR. The interaction of the Ca^{2+} released from the SR with contractile proteins links the heart's electrical activity with mechanical contraction and is termed excitation–contraction coupling (ECC); as such, it is a vital process for cardiac function. Abnormalities in ECC underlie life-threatening arrhythmias in several pathologies ranging from catecholaminergic polymorphic ventricular tachycardia (CPVT) to heart failure (HF)

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(Belevych *et al.* 2013; Radwanski *et al.* 2013*a*). That Ca^{2+} cycling within cardiac myocytes is inextricably intertwined with the regulation of intracellular sodium (Na^+) is well established (Bers *et al.* 2003; Murphy & Eisner, 2009). Voltage-gated Na^+ channels (Na_Vs) permit Na^+ into the cell, resulting in electrical excitation, thereby initiating ECC. The Na^+–Ca^{2+} exchanger (NCX), on the other hand, electrogenically exchanges 1 Ca^{2+} ion for 3 Na^+ ions (Blaustein & Lederer, 1999; Philipson & Nicoll, 2000), creating a direct link between Na^+ influx into the myocytes and Ca^{2+} cycling. This review will focus on the roles of Na_V isoforms, the principal pathways for Na^+ influx into cardiomyocytes, in modulating cardiac Ca^{2+} cycling in health and in disease, with particular emphasis on the spatial integration of Na_Vs with Ca^{2+} cycling proteins to form a larger macromolecular machine.

The interplay between sodium and calcium

Classically, the NCX's role in the cardiac cycle was viewed as the extrusion, from the cardiac myocyte, of all Ca^{2+} entering the cell during ECC. However, since ion transport via NCX is governed by the concentration gradients of Na^+ and Ca^{2+} as well as the membrane potential, it can operate in both forward (3 Na^+ in: 1 Ca^{2+} out) and reverse modes (3 Na^+ out: 1 Ca^{2+} in; reversal potential according to the Nernst equation). As early as 1990, Leblanc and Hume demonstrated that, in the absence of Ca^{2+} entry via voltage-dependent Ca^{2+} channels, Ca^{2+} entry via reverse mode NCX could elicit Ca^{2+} release from the SR (Leblanc & Hume, 1990). Using tetrodotoxin (TTX; 5 μM) to block Na_Vs, they further demonstrated that this Ca^{2+} entry via reverse mode NCX was dependent upon Na^+ entry via Na_Vs: Na^+ entry through Na_Vs elevates subsarcolemmal Na^+ levels, causing NCX to reverse (3 Na^+ out: 1 Ca^{2+} in) and bring in Ca^{2+}, eliciting Ca^{2+} release from the SR. Shortly thereafter, Lipp and Niggli demonstrated Na^+ current (I_{Na})-induced Ca^{2+} transients in guinea pig ventricular myocytes, which were mediated by reverse mode NCX (Lipp & Niggli, 1994). These investigators concluded that Na^+ influx via Na_Vs resulted in a rapid rise in subsarcolemmal [Na^+], causing NCX to operate in reverse mode.

Although these studies demonstrated a link between Na^+ influx via Na_Vs and SR Ca^{2+} release, the question remained as to the physiological role of I_{Na}-induced Ca^{2+} release. Larbig *et al.* (2010) determined that blockade of I_{Na} with 10 μM TTX decreased the influx of trigger Ca^{2+} resulting in a lower rate of Ca^{2+} release from the SR and a reduced Ca^{2+} transient amplitude in myocytes isolated from wild-type (WT) mice but not in those isolated from NCX knockout mice. These results suggested that Ca^{2+} entry via reverse mode NCX secondary to Na^+ entry via Na_Vs does indeed contribute to activation of SR Ca^{2+} release.

The identity of Na_Vs responsible for the enhanced Ca^{2+} release in the heart is the subject of ongoing research. The predominant Na_V isoform identified in the heart is Na_v1.5 (Chen-Izu *et al.* 2015), which is sensitive to micromolar concentrations of TTX and is therefore, categorized as a TTX-resistant cardiac-type Na_V (cNa_v) (Satin *et al.* 1992). Recent work in the heart (Dhar Malhotra *et al.* 2001; Maier *et al.* 2002, 2004; Westenbroek *et al.* 2013; Radwanski *et al.* 2015), however, has identified the presence of neuronal Na_V isoforms (nNa_vs), so called because they were first identified in neurons. Unlike cNa_Vs (Na_v1.5), nNa_vs (Na_v1.1, 1.3, 1.6) are sensitive to nanomolar concentrations of TTX (Ritchie & Rogart, 1977; Renaud *et al.* 1983). In subsequent work, the groups of Goldhaber and Bridge demonstrated that 100 nM TTX suppressed SR Ca^{2+} release flux and Ca^{2+} transient amplitude in rabbit ventricular myocytes, pointing to a role for TTX-sensitive nNa_Vs in triggering SR Ca^{2+} release (Torres *et al.* 2010). In this vein, our group recently obtained similar findings with 100 nM TTX using optical mapping in intact guinea pig ventricles (Radwanski *et al.* 2013*b*). These results underscore the importance of Na_Vs as modulators of cardiac Ca^{2+} cycling and complement the structural observations that nNavs in cardiac myocytes are localized to invaginations of the surface membrane known as the transverse tubules (t-tubules; Fig. 1; Dhar Malhotra *et al.* 2001; Maier *et al.* 2002, 2004; Westenbroek *et al.* 2013; Radwanski *et al.* 2015) – where Ca^{2+} cycling proteins are also localized. Thus, it has been postulated that, at the beginning of an action potential, they admit Na^+ into areas of closest proximity between the t-tubule sarcolemma and SR. This Na^+ is then extruded out of the cell by the NCX in exchange for Ca^{2+}, 'priming' this nanodomain with Ca^{2+}. This, in conjunction with Ca^{2+} that enters the cell through the L-type Ca^{2+} channels (LCCs), facilities opening of SR Ca^{2+} release channels, ryanodine receptors (RyRs), resulting in a robust Ca^{2+} release and contraction.

Compartmentation of cardiac sodium–calcium cycling: neuronal Na^+ channels in the t-tubules

In both skeletal and cardiac muscle, the dynamics of Ca^{2+} cycling are heavily influenced by the spatial organization of the RyRs, relative to the primary source of trigger Ca^{2+}, LCCs (Fabiato, 1983). Whereas in skeletal muscle, sarcolemmal Ca^{2+} channels (Ca_V1.1) are mechanically linked to RyRs (RyR1), in cardiac muscle, LCCs (Ca_V1.2) and RyRs (RyR2) are closely associated in space without any known direct mechanical linkage. Therefore, the 10–12 nm wide dyadic cleft separating the SR membrane containing the RyRs and the sarcolemma at the t-tubule, containing the LCCs, is of critical importance in determining the functional properties of cardiac Ca^{2+} cycling (Greenstein *et al.* 2006; Koh *et al.* 2006; Cannell

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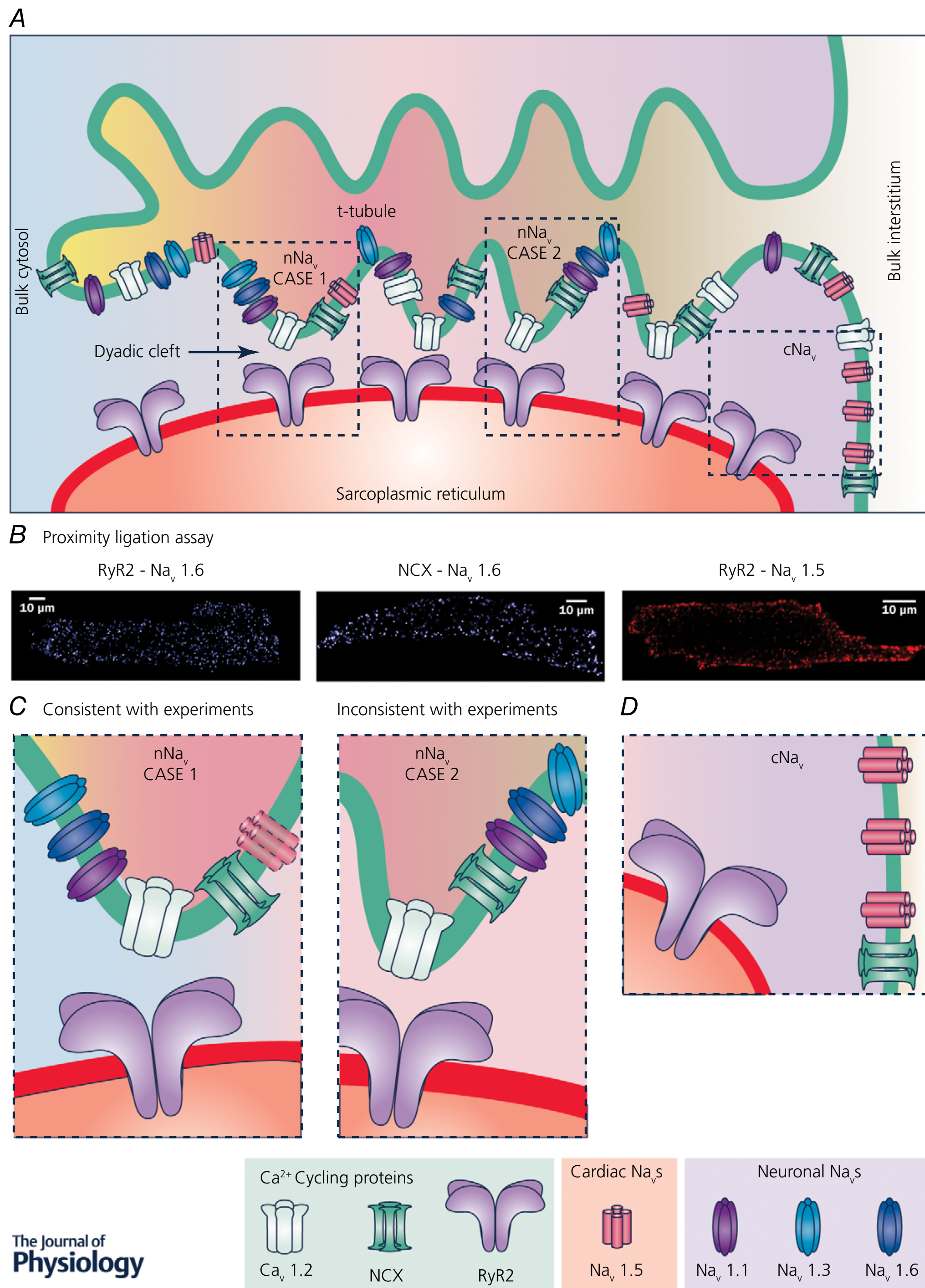

Figure 1. Schematic diagram showing the protein machinery of cardiac Na^+–Ca^{2+} cycling
A, schematic diagram of a t-tubule and associated junctional SR. Microfolds in t-tubule are depicted based on recent findings (Hong *et al.* 2014; Lavorato *et al.* 2015). Different arrangements of Ca^{2+} cycling proteins and sodium channels are depicted along the t-tubule. Regions highlighted by the dashed boxes are presented at higher magnification in *C* and *D*. Note that differential shading of the interstitial space within the t-tubule and the cytoplasm within the dyadic cleft indicates local differences in ionic concentrations within these spaces due to their diffusional isolation from the bulk interstitial space and cytoplasm, respectively. *B*, results from Duolink proximity ligation assays (PLAs) show close association of nNaV isoform Na_V1.6 with both RyR2 and NCX throughout murine

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myocytes, consistent with enrichment of nNa_Vs in t-tubules. In contrast, PLA signal corresponding to association between cNa_V ($Na_V1.5$) and RyR2 is only observed at the periphery of the cell, consistent with cNa_V localization at the lateral membrane. Adapted from Radwański *et al.* (2016). *C*, higher magnification views of regions from *A* showing two possible scenarios of nNa_V localization within t-tubules. Left, case 1, very close association between nNa_Vs and RyRs, which is consistent with PLA results. A cNaV is depicted faded since experimental results including PLA results argue against cNa_V enrichment in t-tubules. Right, case 2, nNa_Vs localized to t-tubules but not very closely associated with RyRs, which is *not* consistent with PLA results. *D*, higher magnification view of region from *A* showing cNa_V ($Na_V1.5$) localization at the lateral membrane.

et al. 2013). In consequence, the spatial organization of Na_vs and NCX relative to RyRs and LCCs is likely to be a critical modulator of Na^+–Ca^{2+} cycling. Further underscoring the importance of the spatial organization of Na^+ and Ca^{2+} cycling proteins relative to each other, Sobie and colleagues demonstrated that LCCs allosterically activate NCX in rabbit cardiomyocytes at positive membrane potentials, thereby enabling further augmentation of trigger Ca^{2+} for SR Ca^{2+} release by Na^+ influx (Sobie *et al.* 2008).

While the presence of Na_Vs within t-tubules is widely accepted, there remains some debate regarding which isoforms are localized there. This question has important implications given the biophysical differences between cNa_Vs and nNa_Vs: the TTX-sensitive nNa_Vs exhibit more positive voltage dependence of gating, i.e. greater channel availability at positive potentials, as well as more rapid activation and inactivation compared to the TTX-resistant cNa_Vs (Fozzard & Hanck, 1996; Maier *et al.* 2004). In a 2000 study, Moore and colleagues, using wide-field microscopy, observed no close association between cNa_Vs and RyRs (Scriven *et al.* 2000). In subsequent work using confocal microscopy, cNa_Vs ($Na_V1.5$) were found to be enriched at the intercalated disk whereas nNa_Vs ($Na_V1.1$, $Na_V1.3$ and $Na_V1.6$) and skeletal muscle Na_Vs ($Na_V1.4$) were enriched within t-tubules (Dhar Malhotra *et al.* 2001; Maier *et al.* 2002, 2004; Westenbroek *et al.* 2013; Radwanski *et al.* 2015). In this context, the aforementioned results highlighting the role of TTX-sensitive Na^+ current in modulating Ca^{2+} release (Larbig *et al.* 2010; Torres *et al.* 2010; Gershome *et al.* 2011; Radwanski *et al.* 2013*b*) argue in favour of nNa_Vs rather than cNa_Vs being a part of the Na^+–Ca^{2+} cycling machinery. However, when interpreting these findings, it is important to bear in mind that the resolution of these microscopy techniques is limited by diffraction to 200–300 nm resolution. Some functional validation was provided by measurements made by Brette and Orchard – these investigators examined Na^+ current densities in both intact and detubulated rat ventricular myocytes and determined that TTX-resistant cNa_Vs ($Na_V1.5$) are predominantly located at the lateral sarcolemma whereas TTX-sensitive nNa_Vs are preferentially localized to the t-tubules (Brette & Orchard, 2006). Furthermore, Lin *et al.* (2011) using cell-attached macropatch demonstrated that cNa_Vs were distributed between the intercalated disk and the midsection of myocytes whereas nNa_Vs were only observed at the latter location. Interestingly, these investigators also observed functional differences in cNa_Vs based on their subcellular location highlighting the role of subcellular location in modulating ion channel function: TTX-resistant Na_vs (likely to be $Na_V1.5$) along the lateral sarcolemma activated at more positive potentials than those at the intercalated disk suggesting that cellular excitability at physiological resting potentials is largely governed by $Na_V1.5$ at the intercalated disk. A related approach to this problem was reported by Bhargava *et al.* (2013) who combined scanning ion conductance microscopy and cell-attached patch clamp to record currents from LCCs as well as clusters of Na_Vs located within t-tubules. However, these investigators used sensitivity to 30 μM TTX to confirm the identity of Na_Vs in their experiments; thus, they did not discriminate between cardiac and neuronal isoforms. It is important to note here that RyRs in cardiac muscle are distributed between the junctional and corbular SR, organized into clusters with uneven gaps (Cabra *et al.* 2016; Franzini-Armstrong, 2016). Thus, it is possible that neuronal and/or cardiac Na_Vs may be present in sufficient density at or near the dyadic cleft interspersed with RyRs to significantly influence Ca^{2+} cycling.

In the context of characterizing t-tubular Na_Vs, novel experimental techniques have proved to be of great value. One such technique is the proximity ligation assay (PLA), which identifies proteins located within 40 nm of each other with extremely high sensitivity (Gullberg & Andersson, 2010). Using PLA in conjunction with confocal immunofluorescence, we recently demonstrated that primarily nNa_Vs ($Na_V1.1$, $Na_V1.3$ and $Na_V1.6$) and, to a lesser extent, cNa_Vs ($Na_V1.5$) are closely associated with RyR2 and NCX in t-tubules in ventricular myocytes isolated from murine hearts (Fig. 1; Radwański *et al.* 2016). Notably, PLA signal is only generated when colabelled proteins are within ~40 nm of each other (Gullberg & Andersson, 2010) suggesting very close association between nNa_Vs and Ca^{2+} cycling proteins (Fig. 1*C* – case 1). And, among the nNa_Vs, $Na_V1.6$ demonstrated the greatest degree of association with Ca^{2+} handling proteins. Overall, these results highlight nNa_Vs and $Na_V1.6$ as potentially important components of nanodomains which comprise the machinery of ECC. However, data from animal models with cardiac-specific

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gene-targeted deletion of nNa_Vs is necessary to confirm this hypothesis.

Physiological modulation of neuronal Na^+ channels

Like cNa_Vs, nNa_Vs possess consensus Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) phosphorylation sites that correspond to DI–II linker (Marionneau *et al.* 2012). Therefore, phosphorylation of nNa_v by CaMKII may account for synergistic interaction between reverse mode NCX and LCC at positive membrane potentials during β-adrenergic stimulation (Viatchenko-Karpinski *et al.* 2005). In fact, recently, we demonstrated CaMKII augmentation of TTX-sensitive nNa_Vs during β-adrenergic stimulation result in an increased intracellular Na^+ influx (Fig. 2*A*, bottom panel) (Radwański *et al.* 2016). The notion of CaMKII regulation of Na^+ influx is consistent with work in genetic models where CaMKII is either rendered constitutively active (Wagner *et al.* 2006) or knocked out (Dybkova *et al.* 2014). Further, there is evidence that augmentation of Na^+ influx through nNa_V in WT murine hearts may be potentially inotropic by increasing Ca^{2+} transient amplitude (Kirchhof *et al.* 2015; Radwanski *et al.* 2015). However, in a setting of altered RyR2 function, augmentation of nNa_V activity may in fact be proarrhythmic (Radwański *et al.* 2016).

Role of neuronal Na^+ channels in cardiac arrhythmias

Abnormal Na_V function underlies arrhythmias in several pathologies (Ruan *et al.* 2009). Over-active Na_Vs in neurons precipitate seizures, whereas in the heart, they can precipitate triggered arrhythmias. As noted above, nNa_V activity is increased during β-adrenergic stimulation in a CaMKII-dependent manner (Radwański *et al.* 2016). Pathologically elevated Na^+ influx into the confined space of the t-tubular junction during late phase 3 of an action potential or during rest (diastole) can precipitate arrhythmias by modulating RyR function via Na^+-dependent signalling mechanisms. For instance, in inherited ryanopathies such as CPVT where RyRs are 'leaky' and the junctional cleft is primed with Ca^{2+}, physiological enhancement of nNa_V activity by isoproterenol precipitated aberrant diastolic Ca^{2+} releases and consequent arrhythmias *in vivo* via an NCX-mediated mechanism (Radwanski *et al.* 2015, 2016). *In silico* studies suggest that pathological accumulation of cytosolic Na^+ and Ca^{2+} facilitate NCX reversal (Armoundas *et al.* 2003; Radwanski & Poelzing, 2011), thereby facilitating aberrant SR Ca^{2+} releases. Such Na^+-mediated signalling increases Ca^{2+} spark frequency through sensitized RyR2 (Radwanski *et al.* 2015). Likewise, in CPVT cardiomyocytes with enhanced SR Ca^{2+} load (via acute, conditional overexpression of SERCA2a), selectively slowing nNa_V inactivation with β-pompilidotoxin increased Na^+ influx, which in turn, through NCX, triggered aberrant Ca^{2+} releases and arrhythmias *in vivo* (Radwanski *et al.* 2015, 2016). Overall, these results point to nNa_Vs as important regulators of aberrant Ca^{2+} release events in such disease states.

Several further examples exist of the arrhythmogenic impact of pathologically increased Na^+ influx via nNa_Vs. In mice lacking the Na^+ channel auxiliary subunit $\beta1$, a compensatory upregulation of a nNa_v isoform, $Na_V1.3$, results in higher rates of aberrant diastolic Ca^{2+} releases in $\beta1$-knockout mice than in their WT littermates (Lin *et al.* 2014). Likewise, a rat pilocarpine-induced epilepsy model evidenced increased persistent Na^+ current secondary to an upregulation of $Na_V1.1$ (Biet *et al.* 2015). This enhanced persistent Na^+ current, in turn, precipitated cardiac arrhythmias *in vivo*. In yet another model of epileptic encephalopathy, gain of function mutation in $Na_v1.6$ resulted in frequent aberrant diastolic Ca^{2+} releases and ventricular arrhythmias upon catecholamine challenge (Frasier *et al.* 2016). Taken together, these results strongly support a role of enhanced persistent Na^+ influx via nNa_Vs, particularly within Na^+–Ca^{2+} cycling nanodomains in precipitating aberrant diastolic Ca^{2+} releases and thereby cardiac arrhythmias (Fig. 2*B*) in multiple pathologies where intracellular (and particularly dyadic) Na^+ and Ca^{2+} is elevated.

Role of neuronal Na^+ channels in the failing heart

Enhanced persistent Na^+ influx is widely acknowledged as a contributor to arrhythmogenesis in acquired forms of ryanopathy, such as heart failure (Valdivia *et al.* 2005; Undrovinas & Maltsev, 2008; Undrovinas *et al.* 2010; Sossalla & Maier, 2012; Antzelevitch *et al.* 2014; Makielski, 2016). Undrovinas and colleagues identified $Na_V1.1$ as a significant contributor to persistent Na^+ influx in a coronary artery embolization-mediated canine model of HF (Mishra *et al.* 2014), highlighting the role of nNa_Vs in this phenomenon. Likewise, NCX function is pathologically enhanced in failing hearts (Pogwizd & Bers, 2002). Taken together, these findings suggest that arrhythmogenic SR Ca^{2+} releases in failing hearts may be triggered by Na^+-driven Ca^{2+} entry. Interestingly, despite the enhanced Na^+ influx and NCX function, failing rat hearts do not demonstrate a synergistic interaction between NCX and LCCs to increase ECC as part of CICR during β-adrenergic stimulation (Viatchenko-Karpinski *et al.* 2005). This may reflect the severe disruption of t-tubules in failing hearts (Li *et al.* 2015), which contributes to compromised mechanical function and reduced functional reserve. Despite this disruption of the t-tubular network in failing hearts, the remaining t-tubules host abnormal functional nanodomains composed of nNa_vs, NCX and hypersensitive RyRs that facilitate temporal synchronization of

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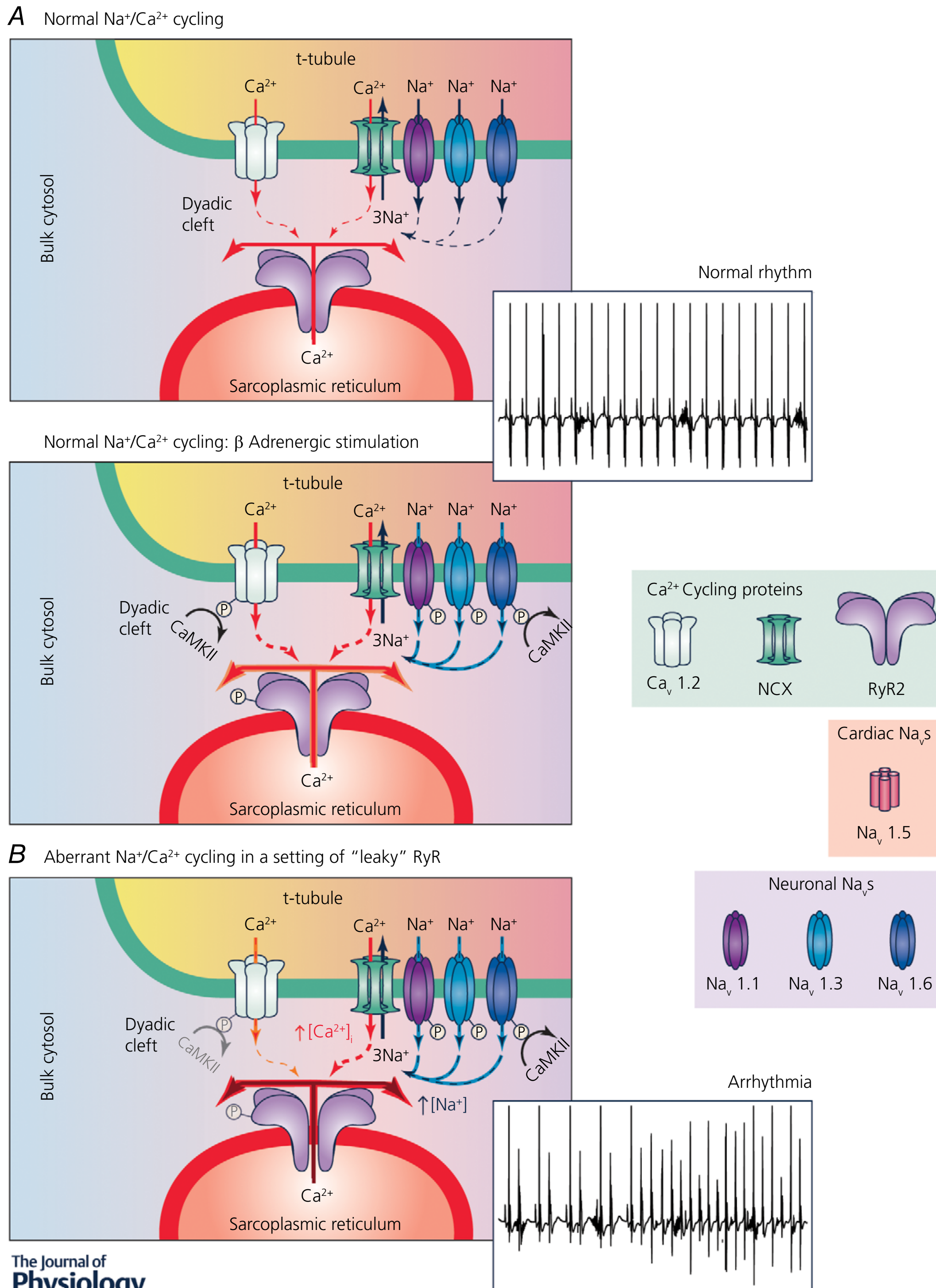

Figure 2. Schematic diagram showing normal and aberrant Na^+–Ca^{2+} cycling
A, top, during normal Na^+–Ca^{2+} cycling, Na^+ influx via nNa$_V$s early in the action potential leads to Ca^{2+} influx via reverse mode NCX, which in turn, in conjunction with Ca^{2+} influx via LCCs, leads to SR Ca^{2+} release via RyRs. Inset shows normal electrocardiogram resulting from normal Na^+–Ca^{2+} cycling. Bottom, during β-adrenergic stimulation, Na^+ influx is enhanced secondary to CaMKII-mediated phosphorylation of nNa$_V$s. This leads to augmented Ca^{2+} influx via reverse mode NCX, which in turn leads to enhanced SR Ca^{2+} release via RyRs. *C*, pathological elevated Na^+ influx via nNa$_V$s results in a larger Ca^{2+} influx via reverse mode NCX. This, particularly in the presence of elevated diastolic Ca^{2+} levels or RyR leak, can trigger arrhythmogenic diastolic Ca^{2+} release. Inset electrocardiogram shows premature beats and arrhythmias triggered by diastolic Ca^{2+} releases.

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aberrant Ca^{2+} release (Belevych *et al.* 2012), thereby precipitating cardiac arrhythmias.

An important debate in this context has been about whether the interplay between Na^+ and Ca^{2+} cycling in the failing heart is mediated by Na^+-driven Ca^{2+} entry via NCX directly triggering SR Ca^{2+} release or by means of this enhanced Ca^{2+} influx being taken up into the SR, thereby enhancing SR Ca^{2+} load. SR Ca^{2+} load in the failing heart is reduced compared to non-failing hearts. This is in line with observations that SR Ca^{2+} load was lowered even when Na^+ entry was enhanced through cardiac glycoside treatment (Ho *et al.* 2011), pointing to Na^+-driven Ca^{2+} entry via NCX directly triggering SR Ca^{2+} release as the likely arrhythmia mechanism. However, this does not exclude a role for elevated SR Ca^{2+} load under other conditions. Thus, structural characterization of Na^+–Ca^{2+} cycling nanodomains in this complex disease state will be crucial to fully understanding this arrhythmia mechanism. Equally, the contribution of nNa_Vs to arrhythmias needs to be investigated in mathematical models incorporating nano-scale structural organization as well as in experimental heart failure models.

Neuronal Na^+ channels as targets for anti-arrhythmic therapy

Given that aberrant diastolic Ca^{2+} releases resulting from altered RyR2 function underlie arrhythmias in a wide range of pathologies, including CPVT(Radwanski *et al.* 2015, 2016) and ischaemic and non-ischaemic cardiomyopathy (Belevych *et al.* 2013), nNa_V inhibition may have wide-ranging therapeutic applications. Such a strategy would also avoid a major pitfall of non-isoform-selective Na_V inhibition: although initially beneficial in managing Ca^{2+}-mediated arrhythmias following myocardial infarction (The CAPS investigators, 1986), the loss of excitability resulting from blunting of peak Na^+ current proved pro-arrhythmic, increasing the incidence of sudden arrhythmic death in patients with structural heart disease (Echt *et al.* 1991; Starmer *et al.* 1991). Since TTX-resistant cardiac Na_Vs ($Na_V1.5$) are primarily responsible for cardiac excitability, selectively targeting nNa_V could beneficially lower pathological persistent Na^+ influx without any detrimental effects on excitability. Indeed, in mice with CPVT, selective inhibition of nNa_Vs with riluzole or 4,9-anhydro-TTX, a TTX analogue, desynchronized pathological diastolic Ca^{2+} releases in both isolated myocytes and in intact tissue, and proved potently antiarrhythmic *in vivo* (Radwanski *et al.* 2015, 2016). Last but not least, selective silencing of $Na_V1.6$ recapitulated this antiarrhythmic effect *in vivo*, suggesting that the therapeutic strategy of inhibiting nNa_Vs could even be further refined to target specific Na^+ channel isoforms.

Future directions: the subcellular milieu of Na^+ channels

The development of novel, selective therapeutic strategies to prevent Ca^{2+}-mediated arrhythmias by targeting nNa_Vs depends on understanding the relationship between structure – the subcellular organization of different nNa_V isoforms and their milieu at the nano-scale, and function – the interplay between Na^+ and Ca^{2+} cycling in the heart. In recent years, we have learned a great deal about the subcellular localization of cNa_Vs. In an elegant 2011 study, Petitprez and colleagues identified two distinct pools of $Na_V1.5$ within cardiac myocytes, one located at the intercalated disk scaffolded by SAP97, and the other located at the lateral sarcolemma scaffolded by the syntrophin–dystrophin complex (Petitprez *et al.* 2011). More recently, we used super-resolution microscopy techniques to identify a subpopulation of intercalated disk-localized $Na_V1.5$ located within 200 nm of connexin43 gap junctions (Veeraraghavan *et al.* 2015; Veeraraghavan & Gourdie, 2016). Importantly, the ultrastructural properties of this juxta-gap junctional membrane region, where the extracellular cleft is just 5–10 nm wide, were shown to be an important modulator of cardiac conduction dependence on the sodium current (Veeraraghavan *et al.* 2015). Likewise, Delmar and colleagues have utilized super-resolution microscopy correlated with electron microscopy to identify yet another subpopulation of intercalated disk-localized $Na_V1.5$ localized to N-cadherin-rich sites, where the membranes of adjacent cells are 50–75 nm apart (Agullo-Pascual *et al.* 2014; Leo-Macias *et al.* 2016). The techniques developed by these investigators could prove very useful in future investigation of the ultrastructural milieu of nNa_Vs in cardiac myocytes. Perhaps more importantly, the identification of these $Na_V1.5$-rich nano-domains suggests that nNa_Vs may also be similarly organized into nano-domains with distinct ultrastructural properties and therefore behave in a location-dependent manner. The hypothesis that the nano-scale milieu of t-tubule-localized Na_Vs may modulate their function is supported by the work of Hong and colleagues: having previously identified a role for the t-tubule protein bridging integrator 1 (BIN1) in Ca^{2+} channel trafficking and clustering at the t-tubule surface (Hong *et al.* 2010, 2012), these investigators recently demonstrated that BIN1 promotes micro-folding of the t-tubular membrane (Hong *et al.* 2014; Fu & Hong, 2016). These microfolds in the t-tubular membrane can trap extracellular ions, thus diffusionally isolating the t-tubular extracellular clefts from bulk extracellular space (Forssmann & Girardier, 1970; Hong *et al.* 2014; Lavorato *et al.* 2015). Importantly, loss of these t-tubular microfolds in heart failure is associated with impaired contractile function secondary to desynchronized ECC as well as elevated risk of ventricular arrhythmias. Not

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only will any nNa_Vs co-distributed with Ca^{2+} handling proteins be subject to intra- and extra-cellular ionic concentrations modulated by local ultrastructure, but studies have also demonstrated that Na_Vs are directly regulated in a Ca^{2+}-dependent manner (Van Petegem *et al.* 2012; Wang *et al.* 2014; Gabelli *et al.* 2016). Therefore, studies of nNa_V localization relative to Ca^{2+}-handling proteins and their local ultrastructural milieu will be critical in understanding the interplay between Na^+ and Ca^{2+} cycling in health and in disease. Further, both the nNa_Vs themselves and the structural proteins that generate their ultrastructural niches could represent valuable therapeutic targets in a wide array of disease states.

Conclusions

In summary, the available evidence suggests that Na^+ influx through Na^+ channels, in particular neuronal Na^+ channels (nNa_vs), contributes both to the triggering of CICR in normal physiology and to arrhythmogenic diastolic Ca^{2+} release during disease states as diverse as CPVT and heart failure. Importantly, selective inhibition of nNa_vs shows promise as a therapeutic strategy in such pathologies, further underscoring the importance of the interplay between cardiac Na^+ and Ca^{2+} cycling. Going forward, understanding the subcellular organization of nNa_vs, particularly in relation to Ca^{2+} handling proteins, will be vital to elucidating the mechanisms underlying this phenomenon as well as to the development of effective therapies against Ca^{2+}-induced arrhythmias.

References

Agullo-Pascual E, Lin X, Leo-Macias A, Zhang M, Liang FX, Li Z, Pfenniger A, Lubkemeier I, Keegan S, Fenyo D, Willecke K, Rothenberg E & Delmar M (2014). Super-resolution imaging reveals that loss of the C-terminus of connexin43 limits microtubule plus-end capture and NaV1.5 localization at the intercalated disc. *Cardiovasc Res* **104**, 371–381.

Antzelevitch C, Nesterenko V, Shryock JC, Rajamani S, Song Y & Belardinelli L (2014). The role of late I Na in development of cardiac arrhythmias. *Handb Exp Pharmacol* **221**, 137–168.

Armoundas AA, Hobai IA, Tomaselli GF, Winslow RL & O'Rourke B (2003). Role of sodium-calcium exchanger in modulating the action potential of ventricular myocytes from normal and failing hearts. *Circ Res* **93**, 46–53.

Belevych AE, Radwanski PB, Carnes CA & Gyorke S (2013). 'Ryanopathy': causes and manifestations of RyR2 dysfunction in heart failure. *Cardiovasc Res* **98**, 240–247.

Belevych AE, Terentyev D, Terentyeva R, Ho HT, Gyorke I, Bonilla IM, Carnes CA, Billman GE & Gyorke S (2012). Shortened Ca^{2+} signaling refractoriness underlies cellular arrhythmogenesis in a postinfarction model of sudden cardiac death. *Circ Res* **110**, 569–577.

Bers DM, Barry WH & Despa S (2003). Intracellular Na^+ regulation in cardiac myocytes. *Cardiovasc Res* **57**, 897–912.

Bhargava A, Lin X, Novak P, Mehta K, Korchev Y, Delmar M & Gorelik J (2013). Super-resolution scanning patch clamp reveals clustering of functional ion channels in adult ventricular myocyte. *Circ Res* **112**, 1112–1120.

Biet M, Morin N, Lessard-Beaudoin M, Graham RK, Duss S, Gagne J, Sanon NT, Carmant L & Dumaine R (2015). Prolongation of action potential duration and QT interval during epilepsy linked to increased contribution of neuronal sodium channels to cardiac late Na^+ current: potential mechanism for sudden death in epilepsy. *Circ Arrhythm Electrophysiol* **8**, 912–920.

Blaustein MP & Lederer WJ (1999). Sodium/calcium exchange: its physiological implications. *Physiol Rev* **79**, 763–854.

Brette F & Orchard CH (2006). Density and sub-cellular distribution of cardiac and neuronal sodium channel isoforms in rat ventricular myocytes. *Biochem Biophys Res Commun* **348**, 1163–1166.

Cabra V, Murayama T & Samso M (2016). Ultrastructural analysis of self-associated RyR2s. *Biophys J* **110**, 2651–2662.

Cannell MB, Kong CH, Imtiaz MS & Laver DR (2013). Control of sarcoplasmic reticulum Ca^{2+} release by stochastic RyR gating within a 3D model of the cardiac dyad and importance of induction decay for CICR termination. *Biophys J* **104**, 2149–2159.

Chen-Izu Y, Shaw RM, Pitt GS, Yarov-Yarovoy V, Sack JT, Abriel H, Aldrich RW, Belardinelli L, Cannell MB, Catterall WA, Chazin WJ, Chiamvimonvat N, Deschenes I, Grandi E, Hund TJ, Izu LT, Maier LS, Maltsev VA, Marionneau C, Mohler PJ, Rajamani S, Rasmusson RL, Sobie EA, Clancy CE & Bers DM (2015). Na^+ channel function, regulation, structure, trafficking and sequestration. *J Physiol* **593**, 1347–1360.

Dhar Malhotra J, Chen C, Rivolta I, Abriel H, Malhotra R, Mattei LN, Brosius FC, Kass RS & Isom LL (2001). Characterization of sodium channel α- and β-subunits in rat and mouse cardiac myocytes. *Circulation* **103**, 1303–1310.

Dybkova N, Wagner S, Backs J, Hund TJ, Mohler PJ, Sowa T, Nikolaev VO & Maier LS (2014). Tubulin polymerization disrupts cardiac beta-adrenergic regulation of late INa. *Cardiovasc Res* **103**, 168–177.

Echt DS, Liebson PR, Mitchell LB, Peters RW, Obias-Manno D, Barker AH, Arensberg D, Baker A, Friedman L, Greene HL *et al.* (1991). Mortality and morbidity in patients receiving encainide, flecainide, or placebo. The Cardiac Arrhythmia Suppression Trial. *N Engl J Med* **324**, 781–788.

Fabiato A (1983). Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. *Am J Physiol Heart Circ Physiol* **245**, C1–C14.

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Forssmann WG & Girardier L (1970). A study of the T system in rat heart. *J Cell Biol* **44**, 1–19.
Fozzard HA & Hanck DA (1996). Structure and function of voltage-dependent sodium channels: comparison of brain II and cardiac isoforms. *Physiol Rev* **76**, 887–926.
Franzini-Armstrong C (2016). Can the arrangement of RyR2 in cardiac muscle be predicted? *Biophys J* **110**, 2563–2565.
Frasier CR, Wagnon JL, Bao YO, McVeigh LG, Lopez-Santiago LF, Meisler MH & Isom LL (2016). Cardiac arrhythmia in a mouse model of sodium channel SCN8A epileptic encephalopathy. *Proc Natl Acad Sci USA* **45**, 12838–12843.
Fu Y & Hong T (2016). BIN1 regulates dynamic t-tubule membrane. *Biochim Biophys Acta* **1863**, 1839–1847.
Gabelli SB, Yoder JB, Tomaselli GF & Amzel LM (2016). Calmodulin and Ca^{2+} control of voltage gated Na^{+} channels. *Channels (Austin)* **10**, 45–54.
Gershome C, Lin E, Kashihara H, Hove-Madsen L & Tibbits GF (2011). Colocalization of voltage-gated Na^{+} channels with the Na^{+}/Ca^{2+} exchanger in rabbit cardiomyocytes during development. *Am J Physiol Heart Circ Physiol* **300**, H300–H311.
Greenstein JL, Hinch R & Winslow RL (2006). Mechanisms of excitation-contraction coupling in an integrative model of the cardiac ventricular myocyte. *Biophys J* **90**, 77–91.
Gullberg M & Andersson A-C (2010). Visualization and quantification of protein-protein interactions in cells and tissues. *Nat Methods* **7**, 1608.
Ho HT, Stevens SC, Terentyeva R, Carnes CA, Terentyev D & Gyorke S (2011). Arrhythmogenic adverse effects of cardiac glycosides are mediated by redox modification of ryanodine receptors. *J Physiol* **589**, 4697–4708.
Hong TT, Smyth JW, Chu KY, Vogan JM, Fong TS, Jensen BC, Fang K, Halushka MK, Russell SD, Colecraft H, Hoopes CW, Ocorr K, Chi NC & Shaw RM (2012). BIN1 is reduced and Cav1.2 trafficking is impaired in human failing cardiomyocytes. *Heart Rhythm* **9**, 812–820.
Hong TT, Smyth JW, Gao D, Chu KY, Vogan JM, Fong TS, Jensen BC, Colecraft HM & Shaw RM (2010). BIN1 localizes the L-type calcium channel to cardiac T-tubules. *PLoS Biol* **8**, e1000312.
Hong TT, Yang H, Zhang SS, Cho HC, Kalashnikova M, Sun B, Zhang H, Bhargava A, Grabe M, Olgin J, Gorelik J, Marban E, Jan LY & Shaw RM (2014). Cardiac BIN1 folds T-tubule membrane, controlling ion flux and limiting arrhythmia. *Nat Med* **20**, 624–632.
Kirchhof P, Tal T, Fabritz L, Klimas J, Nesher N, Schulte JS, Ehling P, Kanyshkova T, Budde T, Nikol S, Fortmueller L, Stallmeyer B, Muller FU, Schulze-Bahr E, Schmitz W, Zlotkin E & Kirchhefer U (2015). First report on an inotropic peptide activating tetrodotoxin-sensitive, "neuronal" sodium currents in the heart. *Circ Heart Fail* **8**, 79–88.
Koh X, Srinivasan B, Ching HS & Levchenko A (2006). A 3D Monte Carlo analysis of the role of dyadic space geometry in spark generation. *Biophys J* **90**, 1999–2014.
Larbig R, Torres N, Bridge JH, Goldhaber JI & Philipson KD (2010). Activation of reverse Na^{+}-Ca^{2+} exchange by the Na^{+} current augments the cardiac Ca^{2+} transient: evidence from NCX knockout mice. *J Physiol* **588**, 3267–3276.
Lavorato M, Huang TQ, Iyer VR, Perni S, Meissner G & Franzini-Armstrong C (2015). Dyad content is reduced in cardiac myocytes of mice with impaired calmodulin regulation of RyR2. *J Muscle Res Cell Motil* **36**, 205–214.
Leblanc N & Hume JR (1990). Sodium current-induced release of calcium from cardiac sarcoplasmic reticulum. *Science* **248**, 372–376.
Leo-Macias A, Agullo-Pascual E, Sanchez-Alonso JL, Keegan S, Lin X, Arcos T, Feng Xia L, Korchev YE, Gorelik J, Fenyo D, Rothenberg E & Delmar M (2016). Nanoscale visualization of functional adhesion/excitability nodes at the intercalated disc. *Nat Commun* **7**, 10342.
Li H, Lichter JG, Seidel T, Tomaselli GF, Bridge JH & Sachse FB (2015). Cardiac resynchronization therapy reduces subcellular heterogeneity of ryanodine receptors, T-tubules, and Ca^{2+} sparks produced by dyssynchronous heart failure. *Circ Heart Fail* **8**, 1105–1114.
Lin X, Liu N, Lu J, Zhang J, Anumonwo JM, Isom LL, Fishman GI & Delmar M (2011). Subcellular heterogeneity of sodium current properties in adult cardiac ventricular myocytes. *Heart Rhythm* **8**, 1923–1930.
Lin X, O'Malley H, Chen C, Auerbach D, Foster M, Shekhar A, Zhang M, Coetzee W, Jalife J, Fishman GI, Isom L & Delmar M (2014). Scn1b deletion leads to increased tetrodotoxin-sensitive sodium current, altered intracellular calcium homeostasis and arrhythmias in murine hearts. *J Physiol* **593**, 1389–1407.
Lipp P & Niggli E (1994). Sodium current-induced calcium signals in isolated guinea-pig ventricular myocytes. *J Physiol* **474**, 439–446.
Maier SK, Westenbroek RE, McCormick KA, Curtis R, Scheuer T & Catterall WA (2004). Distinct subcellular localization of different sodium channel α and β subunits in single ventricular myocytes from mouse heart. *Circulation* **109**, 1421–1427.
Maier SK, Westenbroek RE, Schenkman KA, Feigl EO, Scheuer T & Catterall WA (2002). An unexpected role for brain-type sodium channels in coupling of cell surface depolarization to contraction in the heart. *Proc Natl Acad Sci USA* **99**, 4073–4078.
Makielski JC (2016). Late sodium current: A mechanism for angina, heart failure, and arrhythmia. *Trends Cardiovasc Med* **26**, 115–122.
Marionneau C, Lichti CF, Lindenbaum P, Charpentier F, Nerbonne JM, Townsend RR & Mérot J (2012). Mass spectrometry-based identification of native cardiac Nav1.5 channel α subunit phosphorylation sites. *J Proteome Res* **11**, 5994–6007.
Mishra S, Reznikov V, Maltsev VA, Undrovinas NA, Sabbah HN & Undrovinas A (2014). Contribution of sodium channel neuronal isoform Nav1.1 to late sodium current in ventricular myocytes from failing hearts. *J Physiol* **593**, 1409–1427.

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Murphy E & Eisner DA (2009). Regulation of intracellular and mitochondrial sodium in health and disease. *Circ Res* **104**, 292–303.

Petitprez S, Zmoos AF, Ogrodnik J, Balse E, Raad N, El-Haou S, Albesa M, Bittihn P, Luther S, Lehnart SE, Hatem SN, Coulombe A & Abriel H (2011). SAP97 and dystrophin macromolecular complexes determine two pools of cardiac sodium channels Nav1.5 in cardiomyocytes. *Circ Res* **108**, 294–304.

Philipson KD & Nicoll DA (2000). Sodium-calcium exchange: a molecular perspective. *Annu Rev Physiol* **62**, 111–133.

Pogwizd SM & Bers DM (2002). Na/Ca exchange in heart failure: contractile dysfunction and arrhythmogenesis. *Ann N Y Acad Sci* **976**, 454–465.

Radwanski PB, Belevych AE, Brunello L, Carnes CA & Gyorke S (2013*a*). Store-dependent deactivation: cooling the chain-reaction of myocardial calcium signaling. *J Mol Cell Cardiol* **58**, 77–83.

Radwanski PB, Brunello L, Veeraraghavan R, Ho HT, Lou Q, Makara MA, Belevych AE, Anghelescu M, Priori SG, Volpe P, Hund TJ, Janssen PM, Mohler PJ, Bridge JH, Poelzing S & Gyorke S (2015). Neuronal Na^+ channel blockade suppresses arrhythmogenic diastolic Ca^{2+} release. *Cardiovasc Res* **106**, 143–152.

Radwanski PB, Greer-Short A & Poelzing S (2013*b*). Inhibition of Na^+ channels ameliorates arrhythmias in a drug-induced model of Andersen-Tawil syndrome. *Heart Rhythm* **10**, 255–263.

Radwański PB, Ho H-T, Veeraraghavan R, Brunello L, Liu B, Belevych AE, Unudurthi SD, Makara MA, Priori SG, Volpe P, Armoundas AA, Dillmann WH, Knollmann BC, Mohler PJ, Hund TJ & Györke S (2016). Neuronal Na^+ channels are integral components of pro-arrhythmic Na^+/Ca^{2+} signaling nanodomain that promotes cardiac arrhythmias during β-adrenergic stimulation. *JACC Basic Transl Sci* **1**, 251–266.

Radwanski PB & Poelzing S (2011). NCX is an important determinant for premature ventricular activity in a drug-induced model of Andersen-Tawil syndrome. *Cardiovasc Res* **92**, 57–66.

Renaud JF, Kazazoglou T, Lombet A, Chicheportiche R, Jaimovich E, Romey G & Lazdunski M (1983). The Na^+ channel in mammalian cardiac cells. Two kinds of tetrodotoxin receptors in rat heart membranes. *J Biol Chem* **258**, 8799–8805.

Ritchie JM & Rogart RB (1977). The binding of saxitoxin and tetrodotoxin to excitable tissue. *Rev Physiol Biochem Pharmacol* **79**, 1–50.

Ruan Y, Liu N & Priori SG (2009). Sodium channel mutations and arrhythmias. *Nat Rev Cardiol* **6**, 337–348.

Satin J, Kyle JW, Chen M, Bell P, Cribbs LL, Fozzard HA & Rogart RB (1992). A mutant of TTX-resistant cardiac sodium channels with TTX-sensitive properties. *Science* **256**, 1202–1205.

Scriven DR, Dan P & Moore ED (2000). Distribution of proteins implicated in excitation-contraction coupling in rat ventricular myocytes. *Biophys J* **79**, 2682–2691.

Sobie EA, Cannell MB & Bridge JH (2008). Allosteric activation of Na^+-Ca^{2+} exchange by L-type Ca^{2+} current augments the trigger flux for SR Ca^{2+} release in ventricular myocytes. *Biophys J* **94**, L54–L56.

Sossalla S & Maier LS (2012). Role of ranolazine in angina, heart failure, arrhythmias, and diabetes. *Pharmacol Ther* **133**, 311–323.

Starmer CF, Lastra AA, Nesterenko VV & Grant AO (1991). Proarrhythmic response to sodium channel blockade. Theoretical model and numerical experiments. *Circulation* **84**, 1364–1377.

The CAPS investigators (1986). The Cardiac Arrhythmia Pilot Study. *Am J Cardiol* **57**, 91–95.

Torres NS, Larbig R, Rock A, Goldhaber JI & Bridge JH (2010). Na^+ currents are required for efficient excitation-contraction coupling in rabbit ventricular myocytes: a possible contribution of neuronal Na^+ channels. *J Physiol* **588**, 4249–4260.

Undrovinas A & Maltsev VA (2008). Late sodium current is a new therapeutic target to improve contractility and rhythm in failing heart. *Cardiovasc Hematol Agents Med Chem* **6**, 348–359.

Undrovinas NA, Maltsev VA, Belardinelli L, Sabbah HN & Undrovinas A (2010). Late sodium current contributes to diastolic cell Ca^{2+} accumulation in chronic heart failure. *J Physiol Sci* **60**, 245–257.

Valdivia CR, Chu WW, Pu J, Foell JD, Haworth RA, Wolff MR, Kamp TJ & Makielski JC (2005). Increased late sodium current in myocytes from a canine heart failure model and from failing human heart. *J Mol Cell Cardiol* **38**, 475–483.

Van Petegem F, Lobo PA & Ahern CA (2012). Seeing the forest through the trees: towards a unified view on physiological calcium regulation of voltage-gated sodium channels. *Biophys J* **103**, 2243–2251.

Veeraraghavan R & Gourdie R (2016). Stochastic optical reconstruction microscopy-based relative localization analysis (STORM-RLA) for quantitative nanoscale assessment of spatial protein organization. *Mol Biol Cell* **27**, 3583–3590.

Veeraraghavan R, Lin J, Hoeker GS, Keener JP, Gourdie RG & Poelzing S (2015). Sodium channels in the Cx43 gap junction perinexus may constitute a cardiac ephapse: an experimental and modeling study. *Pflugers Arch* **467**, 2093–2105.

Viatchenko-Karpinski S, Terentyev D, Jenkins LA, Lutherer LO & Gyorke S (2005). Synergistic interactions between Ca^{2+} entries through L-type Ca^{2+} channels and Na^+-Ca^{2+} exchanger in normal and failing rat heart. *J Physiol* **567**, 493–504.

Wagner S, Dybkova N, Rasenack EC, Jacobshagen C, Fabritz L, Kirchhof P, Maier SK, Zhang T, Hasenfuss G, Brown JH, Bers DM & Maier LS (2006). Ca^{2+}/calmodulin-dependent protein kinase II regulates cardiac Na^+ channels. *J Clin Invest* **116**, 3127–3138.

Wang C, Chung BC, Yan H, Wang HG, Lee SY & Pitt GS (2014). Structural analyses of Ca^{2+}/CaM interaction with NaV channel C-termini reveal mechanisms of calcium-dependent regulation. *Nat Commun* **5**, 4896.

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Westenbroek RE, Bischoff S, Fu Y, Maier SK, Catterall WA & Scheuer T (2013). Localization of sodium channel subtypes in mouse ventricular myocytes using quantitative immunocytochemistry. *J Mol Cell Cardiol* **64**, 69–78.

Additional information

Competing interests

None.

Author contributions

R.V., S.G. and P.B.R. contributed equally to the conceptualization of this article. The text was written by R.V. and P.B.R., and revised and edited by all three authors. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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