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SHORT COMMUNICATION

Stretch–excitation correlation in the toad heart

Vladimir A. Vityazev[1] and Jan E. Azarov[1,2,*]

ABSTRACT

The activation sequence of the ventricular myocardium in ectotherms is a matter of debate. We studied the correlation between the ventricular activation sequence and the pattern of local stretches in 13 toads (*Bufo bufo*). Epicardial potential mapping was done with a 56-lead sock array. Activation times were determined as d*V*/d*t* (min) in each lead. Initial epicardial foci of activation were found on the left side of the ventricular base, whereas regions on the apex and the right side of the base demonstrated late activation. Video recordings (50 frames s^{-1}) showed that the median presystolic stretch in left-side ventricular regions was greater than that in right-side regions [4.70% (interquartile range 3.25–8.85%) versus 1.45% (interquartile range 0.38–3.05%), P=0.028, respectively]. Intracardiac bolus injection elicited ventricular activation with a similar sequence and duration. Thus, ventricular areas of earliest activation were associated with greater presystolic stretch, implying the existence of a stretch–excitation relationship in ectotherm hearts.

KEY WORDS: Amphibia, Ventricular myocardium, Mapping, Activation, Stretch

INTRODUCTION

Activation of the ventricular myocardium in mammals is governed by the His-Purkinje system. A depolarization wave from the atrioventricular node enters the conduction fibers, which deliver the excitation impulse through the apical regions of the right and left ventricles to activate the contractile myocardium. Localizations of initial excitation foci in the ventricles usually correspond to the bases of papillary muscles (Azarov et al., 2007) that presumably support the pump function by strengthening valve closure. In avian hearts, the excitation itself and the excitation–contraction relationship are more complex (Kharin, 2004) as a result of the widely distributed conduction fiber terminals (Shmakov et al., 1979) and a probable active role of the right atrioventricular valve in the contraction process (Prosheva et al., 2015). However, a general apex-to-base spread of activation determined by the His-Purkinje fiber distribution is present in avian ventricles, similar to mammals.

In ectotherms, properties of cardiac impulse conduction are less well known, specifically concerning ventricular activation sequences. This uncertainty is important because observations of the apex-to-base or base-to-apex activation sequence may suggest the presence or absence of a ventricular conduction system, respectively. In this regard, obtained data are conflicting. Opposite activation patterns were observed in systematically close species of amphibians and fish (see table 1 in Jensen et al., 2012). For example, in the same species (*Danio rerio*) both apex-to-base (Sedmera et al., 2003) and base-to-apex (Jensen et al., 2012) activation patterns have been observed. Collectively, these findings suggest that activation of the ventricular myocardium in fish and amphibians is variable (concerning initial activation focus), less organized and slower than in mammals.

These considerations imply that the exact location of the ventricular activation breakthrough may be governed by a functional rather than a structural mechanism. During normal activity, fish and amphibian hearts demonstrate a very broad range of working sarcomere and fiber lengths (Shiels and White, 2008), allowing for great distension during long diastole and almost complete emptying at systole. Mechano-electrical feedback is a mechanism that operates when continuous or pulsatile stretches are imposed on the myocardium, and is able to modify cardiac depolarization and repolarization processes (Patrick et al., 2010; Werdich et al., 2012). Specifically, mechanical stretch can directly activate the ventricular muscle in the amphibian heart (Fasciano and Tung, 1999). Here, we evaluated a correlation between the activation pattern of amphibian ventricular epicardium and the distribution of local stretches of the ventricle in order to test whether or not ventricular epicardial activation breakthrough is spatially related to the region of predominant presystolic stretch.

MATERIALS AND METHODS

Experiments were performed on adult common toads, *Bufo bufo* (Linnaeus 1758) (n=13, body mass 52–85 g, both sexes), collected in a suburban area near Syktyvkar, Komi Republic, Russia (61°29′N; 50°40′E) in August and September, 2019–2020. The procedures conformed to the Guide for the Care and Use of Laboratory Animals, 8th Edition, published by the National Academies Press (US) 2011, and were approved by the ethics committee of the Institute of Physiology of the Komi Science Centre, Ural Branch of the Russian Academy of Sciences. The toads were immersed in an aqueous alcohol solution (20%) for 4–8 min until confirmed unresponsive and were quickly double-pithed by an experienced operator (V.A.V.) with care taken to minimize bleeding. The ambient temperature during the experiments was 20–24°C. The animals demonstrated sinus rhythm with a heart rate of 42±5 beats min^{-1}. The chest cavity was opened and the heart was exposed from the pericardial sac.

Methods of potential contact mapping in the amphibian heart were described previously (Azarov et al., 2007). To briefly summarize here, ventricular epicardial electrograms were led from a sock array of 56 electrodes secured on rubber threads (interelectrode distance 0.5–2.0 mm) with reference to Wilson's central terminal and stored by a custom-designed recording system (144 channels, 16 bits, bandwidth 0.05–1000 Hz, sampling rate 4000 Hz). Activation times (ATs) were measured from the onset of QRS complex to an instant of d*V*/d*t* (min) during the QRS complex. To construct activation maps, the instant of the earliest epicardial activation breakthrough was taken as the zero point.

In order to test whether or not stretch of the ventricle can induce normal ventricular excitation, we injected a saline bolus into the

[1]Department of Cardiac Physiology, Institute of Physiology, Komi Science Center, Ural Branch, Russian Academy of Sciences, Syktyvkar, 167982, Komi Republic, Russia. [2]Department of Biochemistry and Physiology, Institute of Medicine of the Pitirim Sorokin Syktyvkar State University, Syktyvkar, 167001, Komi Republic, Russia.

*Author for correspondence (j.azarov@gmail.com)

V.A.V., 0000-0001-5095-963X; J.E.A., 0000-0003-1779-273X

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ventricle via a catheter introduced in the apical part of the heart (Movie 1). The bolus volume was selected in such a way as to overcome saline leakage and provide sufficient stretch. In five toads, epicardial mapping was performed for normal and bolus-induced QRS complexes following each other. A larger epicardial sock electrode array was applied for this purpose.

In five toads, a pattern of presystolic stretches was assessed by video recording. The heart was accessed so as to ensure a clear view of the anterior surface of the ventricle and the left atrium. The color video was filmed using a Canon 7D (18.0 Megapixel matrix) camera and a macro lens with a focal length of 100 mm. The video recording resolution was 1280×720 pixels, with a frame rate of 50 frames s^{-1}. One cardiac cycle lasted between 63 and 86 frames. Framing was performed using Media Player Classic, version 1.6.8.7400. The image format was JPG with the original video recording resolution. Frame-by-frame processing was performed using JMicroVision 1.2.7. Linear measures of the ventricle for each frame were taken after calibration. The measurements were taken over the anterior surface of the ventricle with the aid of anatomical landmarks and small angular paper markers attached to the epicardial surface.

Statistical analysis was performed using the SPSS package (IBM SPSS Statistics 23) with non-parametric Wilcoxon tests in order to compare activation times and parameters of presystolic stretches on the left and right sides of the ventricle as well as in the normal and bolus-induced excitation. Differences were considered significant at $P<0.05$.

RESULTS AND DISCUSSION

Epicardial contact potential mapping (Fig. 1A,B) showed an activation pattern with a relatively simple activation spread from the left side of the ventricular base to the rest of the ventricular surface. In all studied toads, the left basal region was the first to be activated ($P<0.001$). However, regions of last activation varied in different animals, but most frequently were those most distant from

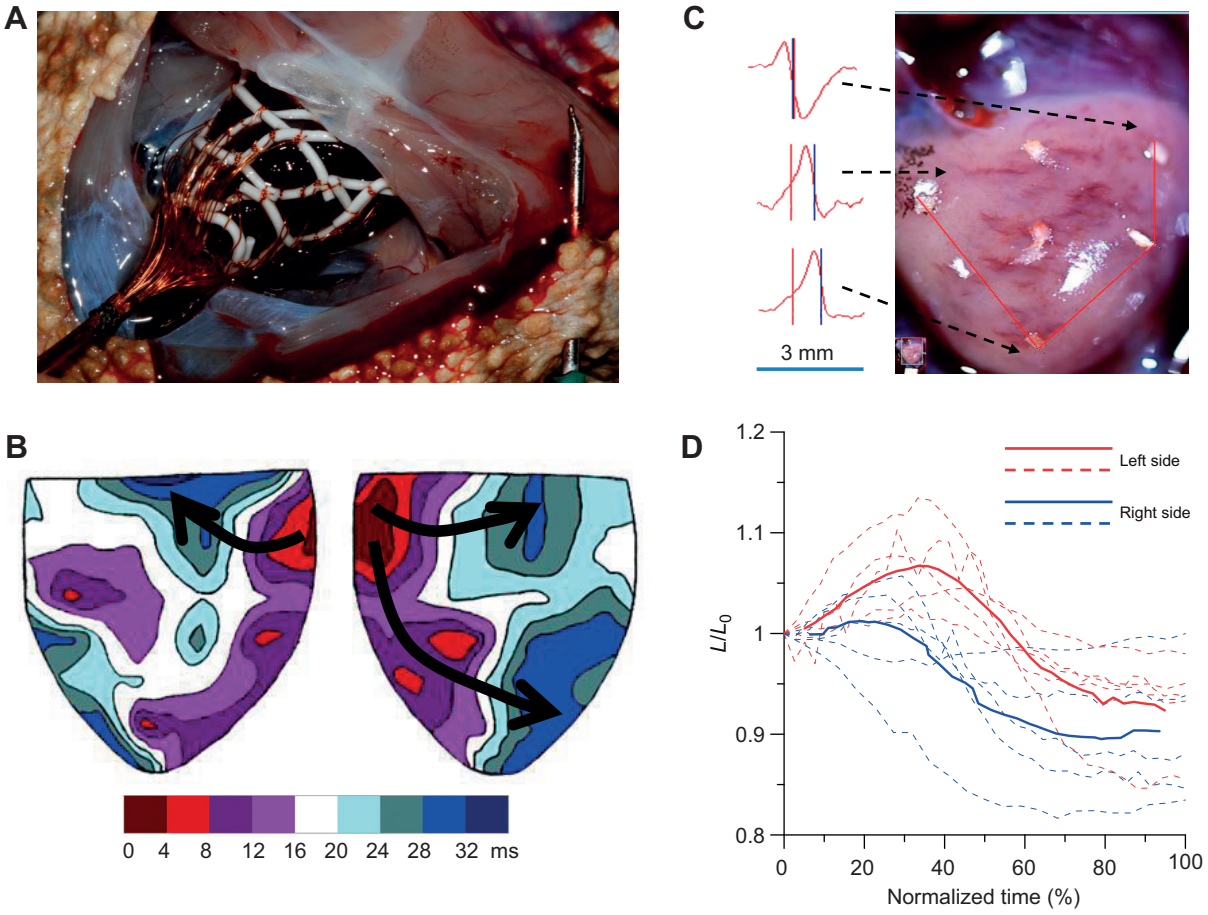

Fig. 1. Electrophysiological and mechanical mapping. (A) A photograph depicting a 64-lead sock array placed on the beating heart ventricle. Lead terminals are the cooper wire knots secured on rubber threads. One limb lead terminal is visible in the bottom right-hand corner. (B) A representative map of the ventricular epicardial activation sequence. Left and right panels represent anterior and posterior surfaces of the ventricle, respectively. The scale shows times of local activation with respect to the earliest epicardial breakthrough. See the area of early activation on the left side of the ventricle (dark red) and the area of late activation on the heart apex, mostly on the right side (blue). (C) A photograph of the anterior ventricular surface with white point markers used for displacement measurements. The stretch was measured on the left and right sides of the ventricle between the appropriate markers along the red lines drawn from the apex to the base on both sides. Representative local unipolar electrograms (QRS complexes) recorded in the basal and apical regions (black arrows) are presented on the left. In the electrograms, the vertical blue and red markers indicate the local activation time and the time of epicardial activation breakthrough, respectively. See early activation in the left base (upper electrogram, the blue and red markers coincide) and late activation on the right side of the ventricle (middle and bottom electrograms). (D) Individual (dotted lines, $n=5$) and averaged (solid lines) graphs of lengthening/shortening of the distance between the corresponding markers (L) with respect to the initial distance prior to atrial contraction (L_0). As cardiac cycle length differed in individual hearts, and diastole duration was relatively long, only the periods of atrial and ventricular contraction are depicted. For clarity, time is normalized with respect to systole duration. See distinct stretch phases (L/L_0 increase) corresponding to atrial contraction for the left side (red), which was much less expressed on the right side (blue).

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the area of ventricular epicardial breakthrough, i.e. on the opposite (right) side of the ventricular base and the heart apex.

The video recordings presented in Fig. S1 show a typical pattern of the ventricular contraction–relaxation cycle. Frame-by-frame analysis showed that the left basal region experienced a distinctly pronounced stretch–shortening amplitude (compare frames 16 and 37). In order to provide a quantitative analysis (Fig. 1C,D), we compared the stretch–shortening patterns along the lines on the left and right sides of the ventricle showing early and late activation, respectively (Fig. 1C). Fig. 1D displays graphs of stretch and shortening of the left and right ventricular areas during atrial and ventricular contractions. The right ventricular region demonstrated slight, if any, stretch during atrial contraction. During ventricular systole, the length of this region shortened as expected. By contrast, the left side of the ventricle showed a distinct stretch phase during atrial contraction and then shortened during ventricular systole.

The left region had a longer dimension by the time of atrial end-diastole (Fig. 2A) and greater lengthening during atrial contraction (Fig. 2B). This more pronounced stretch of the left side was also observed when the stretch was expressed as a proportion of the initial length (Fig. 2C). These data suggest that the crucial factor in this relatively excessive left-side distension was atrial contraction, depicted in Fig. 2D–F.

The above data show that ventricular epicardial activation breakthrough in the toad heart was related to the region that was maximally pre-stretched during atrial contraction. This association does not necessarily mean a cause-and-effect relationship, and in order to substantiate this point we applied a bolus injection into the ventricle in the interbeat period. We did this in order to test: (i) whether such an injection can elicit ventricular activation, and if it can, then (ii) whether the duration of bolus-induced activation differs from the duration of normal excitation and (iii) whether the sequence of bolus-induced activation differs from the sequence of normal excitation. The data from these experiments are presented in Fig. 3 and Movie 1. Injection of the bolus elicited a ventricular excitation with a sequence and – importantly – duration quite similar to the sequence and duration in preceding sinus rhythm complexes with the earliest activation focus at the ventricular base.

In contrast, our previous study demonstrated that electrical pacing at the ventricular base resulted in significant prolongation of activation duration [median 109 ms (interquartile range, IQR 78–165 ms) versus 63 ms (IQR 46–78 ms), $P<0.05$, in ventricular and supraventricular pacing, respectively; Azarov et al., 2013]. Collectively, our findings give us reason to believe that the spatial correlation found between the region of ventricular epicardial activation breakthrough and the region that is maximally pre-stretched during atrial contraction is not a mere coincidence. This correlation implies that stretch may present a natural stimulus facilitating normal ventricular activation. However, the results should be interpreted cautiously considering the limitations of the present study – for example, electrophysiological data were obtained only from extracellular recordings and only on the ventricular epicardial surface.

Generally, there is no consensus on how the activation wave spreads in the ectotherm heart, especially in the ventricle, and marked variation in ventricular activation has been reported. Atrial myocardium normally excites from pacemakers in the sinus venosus, but atrial ectopy is frequently observed (Arbel et al., 1977). Atrioventricular conduction is supposed to be based on the continuity between the chambers (Arbel et al., 1977), specifically the connection between the atrioventricular canal and the ventricular trabecular bands (Sedmera et al., 2003; Icardo and Colvee, 2011) that can function as pathways of conduction to the ventricular myocardium, and variation in morphology of these connections may be responsible for variation in ventricular activation patterns. The ventricular activation sequence of the reptilian heart can change during development depending on the atrioventricular canal's relative position to the growing ventricle (Gregorovicova et al., 2018). The ventricular activation sequence observed in the present study was generally directed from base to apex, consistent

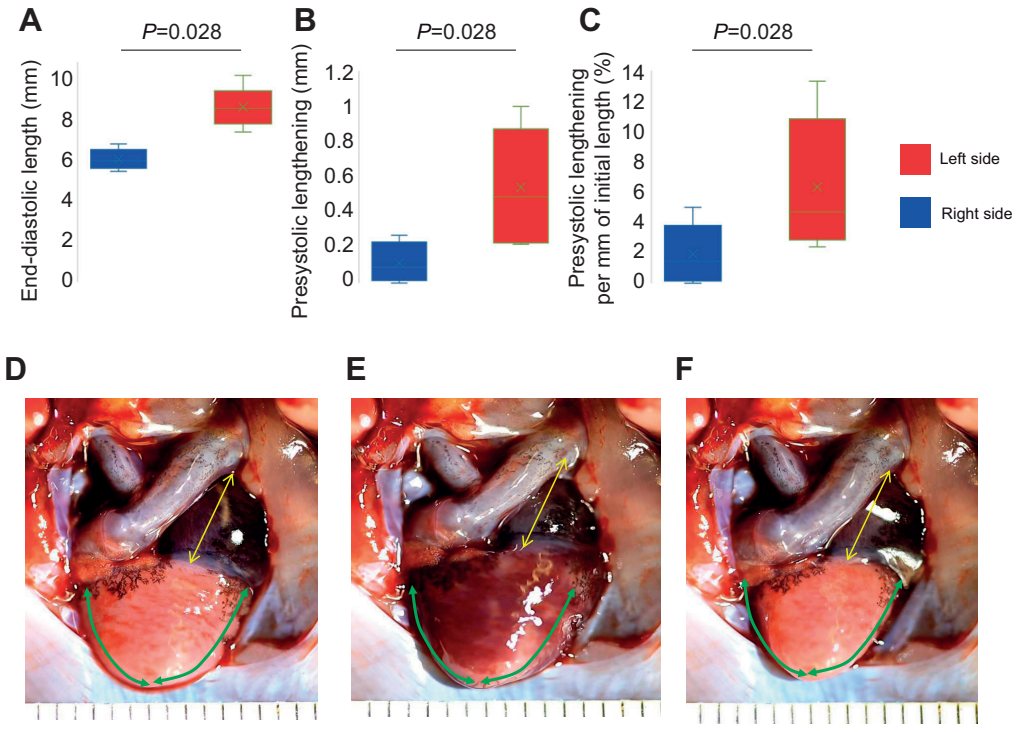

Fig. 2. Non-uniform atrial contraction-related stretch on the left versus right sides of the ventricle. (A–C) Medians (lines), means (crosses), interquartile ranges (boxes) and limits (whiskers) of the end-diastolic (prior to atrial contraction) length (A); absolute presystolic (i.e. related to atrial contraction) lengthening (B); and relative presystolic lengthening expressed as a percentage of the end-diastolic length change (C). (D–F) Frames taken from the video recording of the cardiac cycle displaying relative changes in dimension of the left atrium (yellow double- headed arrow), and left and right sides of the ventricle (green double-headed arrows) prior to atrial contraction (D), during atrial contraction (E) and during ventricular contraction (F). A fragment of calibrated paper (1 mm grid) is presented under the heart apex. Arrow lengths are the same in all three frames. Note the pronounced extension of the left side of the ventricle during atrial contraction (E).

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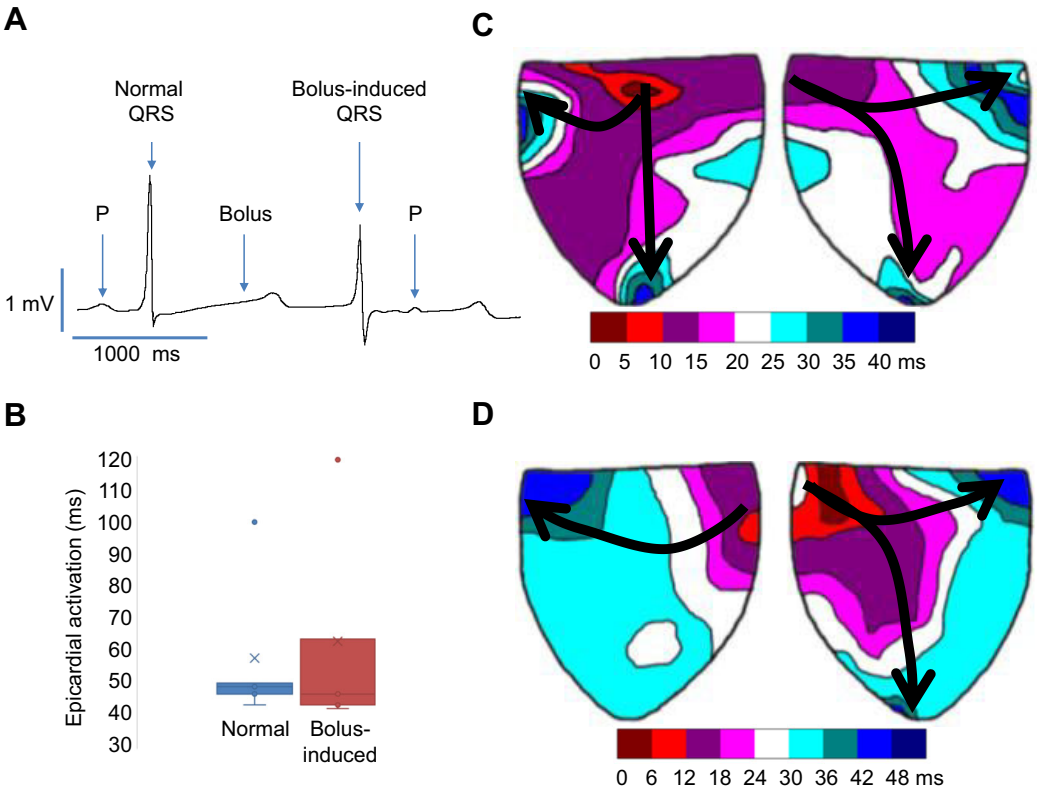

Fig. 3. Comparison of ventricular epicardial activation patterns obtained during two consecutive QRS complexes elicited by normal sinus excitation and by injection of the saline bolus. (A) Electrocardiogram with consecutive normal and bolus-induced QRS complexes. The timing of bolus injection is indicated with an arrow. (B) A box plot [median (line), mean (cross), interquartile range (box) and limits (whiskers)] demonstrating a similar duration of epicardial activation in the normal and bolus-induced ventricular excitations (P=0.465, n=5). (C,D) Representative isochrone maps of epicardial activation in normal (C) and bolus-induced ventricular excitation (D). Bold arrows show the general direction of activation spread. Note the similar base-to-apex and left-to-right activation sequences in the two maps. A shift of the early activation zone in the normal complex (C) is due to loose positioning of the epicardial sock electrode, which was adjusted to match the size of the overstretched ventricle under bolus injection (D). See also Movie 1 for details.

with previously obtained data in fish and amphibian hearts (Azarov et al., 2007; Jensen et al., 2012; Vaykshnorayte et al., 2011). These data support the previous conclusion that ectotherm hearts lack a ventricular conduction system. This conclusion is not universally accepted, as other studies have reported an apex-to-base activation sequence (Chi et al., 2008; Sedmera et al., 2003; Abramochkin and Rozenshtraukh, 2008). Collectively, the data obtained thus far suggest that the ventricular activation pattern in ectotherms is variable and/or non-stable.

Amphibian and fish hearts, especially those with a high proportion of spongy myocardium, can maintain an extremely high ejection fraction (Farrell et al., 2009), which implies functioning in a wide range of sarcomere lengths. This unique capability requires supportive mechanisms in order to prevent contraction impairment at shorter sarcomere lengths (Shiels and White, 2008). One such mechanism is the extension of the ascending limb of a Frank–Starling curve (Shiels and White, 2008). Our study provides another explanation for this contractile effectiveness of the spongy heart – it takes into account the ventricular systole coordination by activation sequence. The ventricular activation sequence builds up the interaction between contractile muscle elements. Fibers in the later-activated regions are set in better conditions according to the Frank–Starling relationship as a result of pre-stretching by the earlier-activated muscle elements. In turn, fibers in the area from which the activation wave experience relatively unfavorable conditions in this interaction (for reviews, see Markhasin et al., 2012; Prinzen and Peschar, 2002). This implies that the pronounced atrial contraction-related stretch of the area of ventricular activation breakthrough facilitates contraction in this region.

In contrast to mammalian hearts, atrial contraction is considered to be a primary factor driving ventricular filling in ectothermic vertebrate hearts (Farrell et al., 2009; Joyce et al., 2018). It is therefore reasonable to posit that the ‘excessive’ stretch of the left basal region was due to filling of the ventricle. However, the proximity of the overstretched region to the atria may indicate a direct mechanical interaction between the atrium and the adjacent ventricular region, thus implying that atrial contraction may modify ventricular electrical activity.

A stretch–excitation association mechanism may be based on the activation of mechano-sensitive channels, e.g. those of the TRP family (Patrick et al., 2010), which can enhance the excitability of the overstretched basal region. Furthermore, atrial contraction-related stretch of the entire left-side ventricular region observed in our study can exceed 10%, which is sufficient to elicit an action potential directly (Fasciano and Tung, 1999). Mechanical stretch can cause transient depolarization, which may shift membrane potential to the threshold level. For example, such transient depolarization was observed in the ventricular myocardium of the zebrafish during atrial activity, whereas the atrial membrane potential lacked this phenomenon (Lin et al., 2014). Another mechanism may be the so-called electrical remodeling of the ventricular myocardium in response to stretch, which can take less than 2 h to evolve and can significantly modify the electrical activity of the stretched region (Werdich et al., 2012). Notably, mapping

studies reporting an apex-to-base activation pattern (opposite to the pattern observed here) were performed using an optical potential recording technique (Abramochkin and Rozenshtraukh, 2008; Chi et al., 2008; Sedmera et al., 2003), where mechanical contraction and hemodynamics are usually significantly altered, if present at all, thus possibly impairing the mechano-electrical feedback in the myocardium and affecting the activation sequence. Whatever the exact mechanism, it is reasonable to speculate that a mechanical stretch – either transient (related to the particular preceding atrial contraction and ventricular filling) or regular – could increase the excitability of the ventricular tissue that provides a direct or supportive mechanism of functional atrioventricular coupling. A recent study (Haverinen and Vornanen, 2020) showed that a decrease of ventricular excitability at high ambient temperatures caused atrioventricular block and ventricular bradycardia, which means that controlling ventricular excitability may be an important mechanism of functional coupling between the chambers.

In conclusion, this is, to our knowledge, the first report of stretch–excitation correlation with regard to the activation pattern in ectotherm hearts. Mechanical intramyocardial interaction may be a previously underestimated factor governing ventricular excitation, and may represent the 'missing link' that reconciles the conflicting cardiac electrophysiological findings obtained in ectotherms to date.

Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.E.A.; Methodology: V.A.V.; Formal analysis: V.A.V., J.E.A.; Investigation: V.A.V., J.E.A.; Writing - original draft: V.A.V., J.E.A.; Writing - review & editing: V.A.V., J.E.A.

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Supplementary information

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