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Fluorescent Imaging of a Dual-Pathway Atrioventricular-Nodal Conduction System

Vladimir Nikolski, Igor R. Efimov

Abstract—A dual-pathway theory to explain atrioventricular-nodal (AVN) reentry has been proposed previously. However, the exact anatomical and functional correlates of the fast pathway (FP) and slow pathway (SP) have not yet been elucidated. We used optical mapping to reconstruct patterns of activation during retrograde conduction through the AVN and during AVN reentry in the triangles of Koch of 12 rabbits. Reentry was inducible by a premature stimulation of the bundle of His in 6 preparations (50%). A functional FP and SP appear to be anatomically correlated with posterior and posterolateral extensions of the AVN, which were recently described. Retrograde breakthrough points in 6 noninducible preparations were clustered near the apex of the triangle of Koch (FP), whereas 6 inducible preparations had either cycle length–dependent FP and SP exits (n = 3) or only SP exits located near the coronary sinus orifice. The shift of breakthrough points from FP to SP during progressive shortening of the coupling interval was accompanied by a discontinuity in the conduction curve. We observed a transmural reentrant circuit involving the AVN, FP, SP, and the superficial endocardial layer of atrial and transitional cells. The presence of a functional SP during retrograde conduction was associated with inducibility of AVN reentry. The full text of this article is available at http://www.circresaha.org. (Circ Res. 2001;88:e23-e30.)

Key Words: ablation ■ electrophysiology ■ arrhythmia ■ imaging

On the basis of functional studies using microelectrodes, Moe et al formulated a theory of a dual-pathway conduction system of the atrioventricular node (AVN) to explain AV-nodal reentry. Yet, the exact anatomical substrate of these functionally described pathways remains unclear. This is due to the difficulties of interpretation of microelectrode and macroelectrode recordings conducted from a small and profoundly complex 3-dimensional structure of the triangle of Koch, which contains the AVN.

The first morphological evidence of extensions in the AVN was presented by Tawara in his seminal study published in 1906. Janse et al later provided an additional description and electrophysiological interpretation of these “blind ending extensions.” Recent morphological studies by Inoue and Becker and Waki et al presented a detailed 3-dimensional structure of the atrioventricular node in the rabbit heart and profoundly complex 3-dimensional structure of the triangle of Koch, which contains the AVN.

The first morphological evidence of extensions in the AVN was presented by Tawara in his seminal study published in 1906. Janse et al later provided an additional description and electrophysiological interpretation of these “blind ending extensions.” Recent morphological studies by Inoue and Becker and Waki et al presented a detailed 3-dimensional structure of the AVN. Unlike microelectrograms, fluorescent signals carry composite information about many layers of cells even if there are no electrotonic interactions between them. Therefore, signatures of several wavefronts propagating asynchronously across different anatomical layers could be deciphered from fluorescent signals. This allows the tracking of the trajectory of impulse propagation during AVN reentry. We applied stimulation at the bundle of His to reveal the points of electric conduction between the AVN and the atrium and to simplify the interpretation of the optical signals from the posterior extension(s).

Materials and Methods

This study conformed to the guidelines of the American Heart Association. We used isolated AVN preparations (see Figure 1A) from 12 rabbit hearts. Preparations were superfused with 37°C Tyrode’s solution containing 15 mmol/L 2,3-butanedione monoxime (BDM). Preparations were paced with a bipolar electrode at the bundle of His. Programmed stimulation was applied, including 20 basic beats with a cycle length of 300 ms followed by a test premature stimulus applied at progressively shorter coupling intervals (300 to 50 ms). Bipolar electrodes at the crista terminalis (CrT), interatrial septum (IAS), and AVN (see Figure 1) were used to track the trajectories of impulse propagation within a complex 3-dimensional structure of the AVN. We applied stimulation at the bundle of His to reveal the points of electric conduction between the AVN and the atrium and to simplify the interpretation of the optical signals from the posterior extension(s).

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construct conduction curves. Preparations were stained by adding to the perfusate di-4-ANEPPS at a final concentration of 1 μmol/L for 40 to 60 minutes. Optical fluorescent signals (F) were recorded from a 5×5- to 8×8-mm area at the triangle of Koch at a rate of 1894 frames/second using a 16×16 photodiode array. A final spatial resolution was 300 to 500 μm per photodiode. The signals were low-pass-filtered at 50 Hz, differentiated (dF/dt), normalized by the basic beat recordings, and plotted as two-dimensional intensity graphs or as animation. Moving bright bands of dF/dt on the basic beat recordings, and plotted as two-dimensional intensity graphs or as animation. Moving bright bands of dF/dt on the animations were considered as a visualization of the propagating action potential wavefronts (see Figures 4 and 6). Using IDL software (Research Systems, Boulder, Colo), we reconstructed 3-dimensional stack-plots, which allowed the visualization of the direction and speed of wavefront propagation in the triangle of Koch as spatiotemporal patterns (see Figure 10). In all preparations, we identified the location of breakthrough points at different coupling directions and speeds of wavefront propagation in the triangle of Koch at a rate of 1894 frames/second using a 16×16 photodiode array. A final spatial resolution was 300 to 500 μm per photodiode. The signals were low-pass-filtered at 50 Hz, differentiated (dF/dt), normalized by the basic beat recordings, and plotted as two-dimensional intensity graphs or as animation. Moving bright bands of dF/dt on the animations were considered as a visualization of the propagating action potential wavefronts (see Figures 4 and 6). Using IDL software (Research Systems, Boulder, Colo), we reconstructed 3-dimensional stack-plots, which allowed the visualization of the direction and speed of wavefront propagation in the triangle of Koch as spatiotemporal patterns (see Figure 10). In all preparations, we identified the location of breakthrough points at different coupling intervals of pacing. Isochronal maps of activation were built as described before, based on the maximums of the first derivatives (dF/dt) max. All quantitative data are expressed as mean±SD.

Results

Voltage-Sensitive Dye Staining

We have previously shown that optical recordings collected at the triangle of Koch during anterograde conduction had two distinct components. The first component corresponded to an atrial-transitional layer of cells and the second component corresponded to the nodal cells. During retrograde conduction, we also observed two components, yet with a reverse sequence, which followed from the reversed temporal sequence of activation. Furthermore, the cellular origin of the two components was revealed during slow staining by superfusion. Figure 1B illustrates the changes in the optical signal morphology recorded during a steady-state pacing at a cycle length of 300 ms at the AV-nodal region (black square dot on Figure 1A) during staining by superfusion. We used 1 μmol/L solution of di-4-ANEPPS, which is 10 to 20 times lower than the previously used concentration. In the first minutes, only the superficial layer of tissue was stained because of the slow diffusion of the dye into deeper layers. As a result, the first component ("hump") of the optical trace, which is supposed to represent the deeper AVN structure during retrograde conduction, became distinguishable only after 20 minutes, whereas the second component appeared after 5 to 10 minutes. Figure 1C shows the time graph of conduction delays measured with bipolar electrodes and amplitudes of the two humps of the optical signal during and after staining. The delivery of the dye was stopped at 40 minutes. The amplitude of the first hump continued to grow because of the maintained diffusion of the dye from the superficial to deeper layers. Meanwhile, the difference between the second and the first hump started to decrease, reflecting dye washout from the surface.

Optical Signal Morphology

Figure 2 illustrates signal morphology during retrograde conduction. Pacing stimuli were applied at the bundle of His, which was within a few millimeters from the right edge of the 6×6-mm field of view. Figure 2A shows a schematic of the entire preparation, field of view (box) and 4 recording sites selected along the hypothetical main axis of the AVN. Signals from these sites are shown in Figure 2B. Site 1 is posterior to the AVN. Sites 2 through 4 are presumably within the nodal area. Figure 2B illustrates the last beat, H1A1, in a sequence of a basic pulses with a 300-ms interval and a premature beat, H2A2, evoked at a coupling interval of 100 ms. Two electrograms recorded at the bundle of His and the crista terminalis are shown. Four optical traces are superimposed. With the exception of trace 1, all traces clearly contain double-component action potentials recorded during both the basic and the premature beats. The delay between the two components was increased during propagation of the premature beat compared with the basic beat. Figure 2C shows a likely interpretation of these data. The scheme represents a
vertical cross section of the AVN along the long axis, which connects the location of the four chosen recording sites illustrated in Figure 2A. Each photodiode recorded the average electrical activity from two (AVN and transitional region) or one (transitional region) layers of tissue. Thus, records 2 through 4 had a first component corresponding to the AVN (white structure) and then a second component corresponding to the activation of the superficial layer of atrial and transitional cells (gray structure). The black structure represents the connective tissue separating the atrium from the distal node and the bundle of His.

Figure 3 illustrates the optical signals (Figure 3A) and derivatives (Figure 3B) recorded during a basic beat from the entire field of view, which is shown in Figure 2A. Area, which has two humps in Figure 3B, is selected with shades of gray. More dark regions show the locations where the ratio between amplitudes of the derivatives of the first and the second humps is greater. This area may represent the projection of the AVN and a part of the extension on the surface.

Maps of Retrograde Activation of the AVN
Figure 4 illustrates the activation pattern reconstructed from these data. The maps in Figure 4A are conventional isochronal maps of the conduction. The left map represents the conduction within the AVN and a part of the posterior nodal extension. It was constructed using the first peaks of the derivatives from only dual-humped signals. The right map shows the conduction after the breakthrough, which activated the endocardial surface layer of the AVN region and the rest of the atrium. This map was built using the second peaks of the derivatives.

Figure 2. Optical signal morphology during retrograde conduction. A, Schematics of the preparation show the location of the 4 selected recording sites illustrated later. CrT indicates crista terminalis; IAS, interatrial septum; IVC, inferior vena cava; CS, coronary sinus orifice; FO, foci ovalis; His, bundle of His; and TV, tricuspid valve. B, Optical traces and electrograms recorded during basic (H1H1 = 300 ms) and premature (H1H2 = 100 ms) beats initiated at the bundle of His. Numbers refer to the corresponding recording sites, shown in panel A. C, Schematic cross section of the triangle of Koch along the main axis of the AVN and the posterior extension (see text for details).

Figure 3. Map of optical action potentials, F (A) and their derivatives, dF/dt (B) during retrograde conduction. Optical recordings were collected from a 6×6-mm field of view during retrograde activation of the AVN by a stimulus applied at the bundle of His. Figure 4C shows the field of view. Signals recorded from the anterior part of the triangle of Koch have a dual-humped morphology. Shades of gray represent the ratio between the first and the second peaks of derivative of the signals. The map of this ratio can be considered as an image of the AVN and its posterior extension.
derivatives in dual-humped signals and the only peaks in single-humped signals. This map illustrates that the activation first spread along the elongated structure, hypothetically located below the surface, until it reached a breakthrough point. The wavefront then emerged at the surface of the preparation and rapidly spread out across its entire surface in a radial fashion. The breakthrough point is located in the middle of the triangle of Koch. Unfortunately, this approach has an important limitation. The method does not permit the visualization of the entire posterior nodal extension in most cases, because the optical signals originating from the posterior extension of the AVN are typically buried under a much stronger signal originating from the superficial layer of transitional and atrial cells.

We used an alternative method to obtain additional information. Figure 4D shows snapshots of the first derivative dF/dt of inverted optical signals. Frames are separated by 10 ms. The first two rows of frames illustrate the impulse conduction across the AVN as shown in conventional isochronal maps in Figure 4A. The two left frames of the lower row show the takeoff of the derivative posterior to the AVN. The geometry of this pattern is consistent with the reported posterior extension anatomy. Comparison of these two frames with subsequent frames shows why the posterior bundle could not be visualized with the conventional isochronal map. The presence of an overwhelming wavefront, which originated later at the point of breakthrough but spread much faster and buried the optical signature of the bundle before it reached (dF/dt)max, made it impossible to resolve in a conventional isochronal map.

Figure 5 summarizes the images of the AVN and extensions obtained in this example. Figure 5A shows the image of the AVN obtained by averaging the ratio between the two peaks, as in Figure 3. It represents an area of the triangle of Koch in which clear dual-humped signals were observed. Figure 5B shows the location of a wavefront just before the breakthrough, which provided an anatomical substrate for conduction from the position of wavefront just before the breakthrough. Hypothetically, this is an image of AVN and its extensions.

**Figure 5.** Reconstruction of the AVN and posterior extensions. A, Image of the AVN reconstructed from the ratio of the 2 peaks (see Figure 3). B, Image of the extensions reconstructed from the position of wavefront just before the breakthrough (see 2 left lower images of wavefronts in Figure 4D). C, Combined image of the AVN and the extensions is shown relative to major anatomical landmarks. Arrows show the direction of activation.

**Figure 6.** Maps of anterograde activation of the AVN. A, Isochronal maps of activation. B, Bipolar electrograms showing the sequence of activation, which started from the CrT. C, Instant snapshots of wavefronts of activation (dF/dt) are shown 20 ms apart.

**Anterograde Conduction**

For comparison, Figure 6 illustrates anterograde conduction in the same preparation in response to a single premature stimulus applied at the crista terminalis at a coupling interval of 120 ms. Figure 6A shows traditional isochronal maps, which illustrate the conduction in the atrium activating the entire field of view, followed by the conduction in a well-defined channel. Figure 6C shows the same propagation as a sequence of snapshots of dF/dt, 20 ms apart. The first 3 frames show rapid activation of the atrial layer. Subsequent frames show the formation and propagation of the wavefront within the posterior extension and the AVN itself. The comparison between the retrograde and anterograde conduction illustrates the difficulty in resolving the signature of the posterior extensions during anterograde conduction.

**Figure 7.** Summary of the breakthrough points and conduction curves. A, Anatomical location of the breakthrough points in 12 studied preparations. The circles show breakthrough points in 3 preparations with a significant shift of the point from anterior (FP) to posterior (SP) sites. The squares show locations of retrograde exit points, which are marked with stars, also served as exit points during AVN reentry. In 5 preparations, reentry was inducible only in one direction with intranodal conduction in the retrograde direction and exit near the coronary sinus orifice. In one preparation, it was inducible in both directions. In 6 preparations, which had no evidence of functional SP, reentry could not be induced. B, Conduction curves measured during retrograde conduction in 12 studied preparations. Dotted lines with a “jump” illustrate preparations with a shift of breakthrough from FP to SP exits. Remaining preparations had no shift (see text for detail).
signal from the extensions was sometimes buried in the signal, corresponding to the activation of atrial and transitional layers of tissue. This especially presents a problem during anterograde conduction. Alternatively, such signature could be missed because of the relatively low amplitude compared with the level of noise.

Cycle Length–Independent Breakthrough Points at the Anterior and Middle of the Triangle of Koch Six preparations (50%) had only the fast-pathway (FP) exit from the AVN during retrograde conduction. The breakthrough points were in the middle or at the apex of the triangle of Koch (see Figure 7A). Their conduction curves were relatively smooth and even flat (Figure 7B). No reentry was induced in these preparations. Figure 8 illustrates an example of a stationary breakthrough point, which was located in the middle of the triangle of Koch. Shortening of the coupling interval from 300 to 140 ms resulted in some deterioration of initially well-defined area of breakthrough, yet no significant repositioning was observed.

Shift of Breakthrough Points From the Anterior to Posterior of the Triangle of Koch In 3 other preparations, we observed a significant and easily defined shift of the breakthrough point from the anterior triangle of Koch to the coronary sinus orifice, which was accompanied by a prominent jump in the conduction curve. In all of these preparations, AVN reentry was inducible. Figure 9 illustrates one of the preparations, where the location of the breakthrough point moved from the FP exit point (areas near the anterior corner of the triangle of Koch in Figure 9A) to the SP exit point (area near the coronary sinus orifice).

Summary of the Breakthrough Points Figure 7 shows the summary of breakthrough points and the conduction curves recorded in all 12 studied preparations. In 3 preparations, the breakthrough points (circles) clearly shifted from the anterior area of the triangle of Koch, or the FP exit, to the posterior, or the SP exit on shortening of the premature coupling interval. This shift of the exit point at the coupling interval of 199±25 ms caused a jump of 64±15 ms in the conduction curve, as seen in Figure 7B (dotted lines). In all of these preparations, reentry was inducible (1 to 2 reentrant beats). Exit sites marked with stars show the breakthrough points during reentry. In one preparation, reentry was inducible in both directions (FP and SP exits points), whereas in the remaining two preparations, the reentrant circuits were only unidirectional. Intranodal and SP conduction was retrograde, and extranodal conduction across the superficial layer of atrial and transitional cells and FP was anterograde.

In the remaining 9 preparations, there was no apparent shift in the location of the breakthrough points, which are shown with squares (Figure 7B). Yet, there was a difference among them with respect to inducibility of reentry. In 3 preparations

Figure 8. Stationary breakthrough points during retrograde activation. A, Preparation and location of the breakthrough points during different coupling intervals. B, Conduction curves built during premature stimuli at different coupling intervals, H1H2. C, Isochronal maps of activation after the breakthrough. Areas of earliest activation are selected at coupling intervals of 300, 170, and 140 ms, respectively. As seen from these panels, shortening of the coupling interval from 300 to 140 ms resulted in some deterioration of initially well-defined area of breakthrough, yet no significant repositioning was observed.

Figure 9. Shift of breakthrough point during premature stimuli at different coupling intervals associated with a “jump” in a conduction curve. A, Preparation and sites of breakthrough at different coupling intervals. B, Conduction curves. Notice a significant jump in the curves, especially which corresponds to His-septum conduction delay. C, Isochronal maps of activation after the breakthrough. Areas of earliest activation are selected at coupling intervals of 300, 170, and 140 ms, respectively. As seen from these panels, the location of the breakthrough point moved from the anterior area of the triangle of Koch to the coronary sinus orifice.
(boxes with stars), breakthrough points were closer to the SP compared with the FP. These were inducible preparations with the same exit points during reentry (stars). In contrast, the remaining 6 preparations had stationary breakthrough points near the FP or the middle of the triangle of Koch (empty boxes). AVN reentry could be induced in none of these 6 preparations at any coupling interval of retrograde pacing.

Furthermore, in 7 of these 9 preparations with stationary breakthrough points, the conduction curves appeared smooth and flat (solid lines in Figure 7B). In the remaining 2 of 9 preparations, the curves (dashed lines in Figure 7B) had an apparent jump just before the block of conduction. Yet, no plateau at the shortest coupling intervals was observed after the jump at short prematurities, as it was seen in the 3 preparations with shifted breakthrough points. Analysis of the conduction patterns in these two preparations revealed that this jump resulted from a significant increase of the conduction delay between the pacing site and the apex of the triangle of Koch. The mechanism of this increase in the conduction delay in these two preparations remains unknown.

**Figure 10.** Stack-plot visualization of AVN reentry. Space-time plots of the dual-pathway conduction through the AVN. Data were collected from a square field of view containing the triangle of Koch in the preparation, shown in Figure 1. Plots show 4 subsequent beats, which are also documented in Figure 11. Different heights of the plots correspond to different durations of analyzed time intervals in Figure 11. Three-dimensional volumes were built by stacking the sequentially recorded two-dimensional plots of dF/dt. Then an isosurface was built using a density threshold, which was adjusted with time to preserve the continuity of conduction along the pathways. White ellipses show points of entry into the plot and points of exit from the 3-dimensional plot. A, FP (right branch) retrograde conduction during the basic beat. B, SP (left branch) retrograde conduction during the premature beat at a coupling interval of 160 ms. C, AVN reentry beat involving both the FP and the SP. D, FP anterograde conduction during self-termination of reentry. See text for detail.

**Figure 11.** Bipolar electrograms recorded during AVN reentry. Recording sites are shown in Figures 1 and Figure 10A. Time intervals selected correspond to stack-plots A-B-C-D in corresponding panels of Figure 10. Top trace shows bipolar electrogram recorded from the apex of the triangle of Koch. It carried the following responses: A, Basic beat. AVN electrogram carries signatures of the His bundle activation (1), AVN activation (2), the onset of the FP signature (3), followed and overwhelmed by the response of the atrial-transitional layer (4). Notice in the two traces below that IAS activation precedes CrT activation, because of the FP breakthrough. B, Premature beat. AVN carries signatures of the His bundle (5), the AVN (6), the dying FP (7), and the atrial-transitional layer (8). Notice the reversal of the CrT-IAS sequence caused by the switch of the breakthrough site from fast to slow. C, Reentry beat. AVN carries signatures of the FP and the AVN (9), the bundle of His (10), and the atrial-transitional layer (11). CrT-IAS sequence is maintained. D, Termination of the AVN reentry in the SP, AVN carries signatures of the FP and the AVN (12) and the bundle of His (13). Notice lack of CrT and IAS activations.

**Stack-Plot Visualization of AVN Reentry**

In 6 of 12 (50%) preparations, we observed reentry in response to retrograde premature stimulation. Fluorescent imaging revealed the reentrant circuit involved in this arrhythmia. We used two methods to visualize the reentry: animation and 3-dimensional stack-plots. Figures 10 and 11 and the online-only movie (data supplement available at http://www.circresaha.org) illustrate reentry in one of the preparations. The 3-dimensional stack-plots A-B-C-D in Figure 10 show impulse propagation during the corresponding time intervals shown in Figure 11.

During the basic beat (Figures 10A and 11A) an impulse entered the AVN from the His bundle and split into two wavelets. One propagated rightward, toward the tendon of Todaro (FP exit), whereas the other propagated leftward, along the posterior nodal extension. Conduction via the FP reached the breakthrough point earlier and rapidly activated the atrium, which annihilated the SP impulse.

During a premature beat (Figures 10B and 11B) applied at a coupling interval of 160 ms, the FP was still refractory, and as a result, conduction went through the SP and rapidly activated the atrium. Reduced amplitude of the FP bipolar electrogram during a premature beat (response 7 in Figure 11B) relative to the basic beat (response 3 in Figure 11A) is consistent with the idea of decremental conduction, which provided reduced and apparently insufficient driving force to activate the atrial layer of cells.
During slow propagation along the SP, the blocked FP was able to fully recover. Therefore, after breakthrough from the SP exit and activation of the entire atrial surface layer, excitation reentered the AVN through the FP (see white ellipse at the top plane of Figure 10C). It then split into two wavelets. One wavelet went rightward through the AVN and left the field of view toward the bundle of His (another white ellipse in Figure 10C). The optical signature of His activation was synchronous (see the online-only movie in the data supplement) with a bipolar response (10 in Figure 11C). At the same time, the other leftward wavelet spread across the SP, again reaching the SP breakthrough point on the surface of the atrium. After rapid activation of the atrium, the wave again reentered the FP (white ellipse in Figure 10D) and split into two. Once again, one wavelet crossed the AVN and exited toward the bundle of His (ellipse in Figure 10D) synchronously with the bipolar waveform (13 in Figure 11D). The other wavelet that reentered the SP terminated quickly. Therefore, the reentry was self-terminated. Unlike human or canine hearts, the rabbit heart rarely supports sustained AVN reentrant tachycardia. In our experiments, we never observed more than two reentry beats.

Discussion
AV-nodal conduction has fascinated both basic and clinical electrophysiologists for nearly a century. A large body of literature has been accumulated by several generations of investigators. Recent clinical success in treatment of AV-nodal reentrant tachycardia by radiofrequency ablation of the SP has rejuvenated interest in the structural-functional relationship of the AVN and the triangle of Koch. Yet, despite the efforts of many investigators, the mechanisms of conduction through the AVN remain unclear. A recent comprehensive work by Mazgalev and Tchou provides an update in the field. The main obstacle impeding the efforts of investigators is the lack of experimental techniques capable of measuring a 3-dimensional pattern of activation in the AVN. Novel imaging techniques may prove to be a key to unlock this century-old puzzle. Application of fluorescent imaging with voltage-sensitive dyes represents the first step in this direction.

Our study presents the first functional fluorescent visualization of the dual-pathway conduction in the AVN of the rabbit heart during retrograde activation and AV-nodal reentry/ventricular echo. In the present study, we show that AVN reentry, observed during ventricular echo beat, involves the AVN itself, the FP and the SP, as well as the superficial layer of atrial and transitional cells enveloping the AVN and its posterior and posterolateral approaches. Visualization of the activation propagation as a sequence of dF/dt intensity plots or animations helps to clarify the relationship of the AVN and the triangle of Koch. Yet, despite the advances methods such as two-photon fluorescence or optical coherent tomography could potentially resolve the signals collected from the different depths of the preparation.

To eliminate the movement artifact during optical recording, we had to use BDM, which could affect AV-nodal conduction and ventricular echo inducibility. Preparation staining with di-4-ANEPPS could also potentially produce similar side effects.

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Online Figure 1. Animation of the AV-nodal reentry. Scheme of the preparation. A scheme of the preparation with the marked positions of the recording electrodes and the field of view, which corresponds to Online Movie 1. Abbreviations: CrT - crista terminalis, IAS - interatrial septum, IVC - inferior vena cava, CS - coronary sinus orifice, FO - foci ovalis, His - bundle of His, TrV - tricuspid valve.

Online Movie 1. Spread of activation and reentry at Koch triangle in one of the preparations. Animation of the data is shown in the figures 10,11. Optical action potentials were differentiated in order to show the spread of wavefronts of action potentials and visualize the reentry. Frames were taken 2.5 ms apart. A yellow rectangle on a scheme of the preparation (Online Data Supplement Figure 1) shows the field of view. Bipolar electrode positions are marked with color circles at Crista Terminalis (CrT), Intra-atrium Septum (IAS), AN-node (AVN), and His bundle (His). Recording from these electrodes with a time cursor are shown below the movie of optical signal derivatives. A pacing stimulus was applied at the His (red) electrode. During the basic 300 ms beat conduction was via the fast pathway. During the premature beat applied at a coupling interval of 160 ms, the fast pathway was still refractory, and as a result conduction went through the slow pathway and rapidly activated the atrium. During this propagation along the slow pathway, the fast pathway was able to fully recover. Therefore, after reaching the fast pathway approach to the AVN via an atrial layer, an excitation wavelet entered into it and split into two wavelets. One wavelet went to the AVN and exited the field of view toward the bundle of His. At the same time, the other wavelet spread along the slow pathway, reaching the breakthrough on the surface of the atrium. Following rapid activation of the atrium, the wave again reentered the fast
pathway and split into two. Once again one wavelet crossed the AVN and exited toward the bundle of His. The other wavelet vanished. Therefore, the reentry was self-terminated.
Animation of the AV-nodal reentry
Scheme of the preparation

CrT  IVC  IAS  FO

CS  AVN  tT  TrV  His

1 mm