Mechanisms Underlying the Reentrant Circuit of Atrioventricular Nodal Reentrant Tachycardia in Isolated Canine Atrioventricular Nodal Preparation Using Optical Mapping

Jianyi Wu, Jiashin Wu, Jeffrey Olgin, John M. Miller, Douglas P. Zipes

Abstract—The reentrant pathways underlying different types of atrioventricular (AV) nodal reentrant tachycardia have not yet been elucidated. This study was performed to optically map Koch’s triangle and surrounding atrial tissue in an isolated canine AV nodal preparation. Multiple preferential AV nodal input pathways were observed in all preparations (n=22) with continuous (73%, n=16) and discontinuous (27%, n=6) AV nodal function curves (AVNFCs). AV nodal echo beats (EBs) were induced in 54% (12/22) of preparations. The reentrant circuit of the slow/fast EB (36%, n=8) started as a block in fast pathway (FP) and a delay in slow pathway (SP) conduction to the compact AV node, then exited from the AV node to the FP, and rapidly returned to the SP through the atrial tissue located at the base of Koch’s triangle. The reentrant circuit of the fast/slow EB (9%, n=2) was in an opposite direction. In the slow/slow EB (9%, n=2), anterograde conduction was over the intermediate pathway (IP) and retrograde conduction was over the SP. Unidirectional conduction block occurred at the junction between the AV node and its input pathways. Conduction over the IP smoothed the transition from the FP to the SP, resulting in a continuous AVNFC. A “jump” in AH interval resulted from shifting of anterograde conduction from the FP to the SP (n=4) or abrupt conduction delay within the AV node through the FP (n=2). These findings indicate that (1) multiple AV nodal anterograde pathways exist in all normal hearts; (2) atrial tissue is involved in reentrant circuits; (3) unidirectional block occurs at the interface between the AV node and its input pathways; and (4) the IP can mask the existence of FP and SP, producing continuous AVNFCs. (Circ Res. 2001;88:1189-1195.)

Key Words: atrioventricular node ■ atrioventricular nodal reentrant tachycardia ■ optical mapping

Materials and Methods

AV Nodal Preparation

The AV nodal preparations used in the present study were modified from canine atrial preparation described previously.7 Adult mongrel dogs were anesthetized. Hearts were rapidly excised and perfused through the aorta with cardioplegic solution. After the ventricle, left atrium, right atrial appendage, and sinoatrial node tissues were trimmed away, the proximal and distal right coronary artery and distal left circumflex artery were separately cannulated. The preparation was perfused with Tyrode’s solution at 37°C through the coronary cannulae at a flow rate of 20 mL/min.

Fluorescent Optical Mapping System and Data Processing

The optical mapping system was constructed as described previously.5,9 The locations of the mapped area (19.5×19.5 mm) were verified using a CCD video camera as shown in Figure 1A. A bipolar electrode was used to record His bundle electrograms. Position was performed from the anterior limbus of the fossa ovalis near the fast pathway (FP) (Figure 1A). The fluorescent action potential (AP) signals were filtered at 1000 Hz. The time of activation was verified using a CCD video camera as shown in Figure 1A. A bipolar electrode was used to record His bundle electrograms. Pacing was performed from the anterior limbus of the fossa ovalis near the fast pathway (FP) (Figure 1A). The fluorescent action potential (AP) signals were filtered at 1000 Hz. The time of activation was determined from the maximum amplitude of the APs (APA-max). The benefits and limitations of this method are discussed in an online data supplement available at http://www.circresaha.org. The conduc-
A picture of an AV nodal preparation is shown in Figure 1A. The FP was identified as the earliest retrograde atrial activation sites located near the anterior atrial septum outside Koch’s triangle (KT). The slow pathway (SP) region between the coronary sinus (CS) ostium and the tricuspid annulus was divided equally into 3 zones (Figure 1B). Optical APs recorded during atrial pacing at 800 ms are shown in Figure 1C. Representative optical and intracellular APs obtained from the atrium, FP, transitional zone, AV node, and SP are superimposed and displayed in Figure 1D. Each optical AP represents a voltage signal summated from many cells. The optical AP duration (APD) was expected to be longer than the intracellular APD as shown in Figure 1D, especially in the optical mapping area. Sites of microelectrode recording the His bundle electrogram (H) are located in top portion of the picture. Outlined area next to tricuspid valve (TV) represents the optical mapping area. B, Enlarged picture of optical mapping area. C, Optical APs recorded from the same preparation. For clarity, only alternate APs are shown. D, Optical (top) and intracellular APs recorded from different anatomic regions are superimposed and displayed at fast sweep speed. The horizontal dashed lines represent the level of 0 mV for each intracellular AP. The vertical dashed line indicates the beginning of atrial pacing at cycle length of 800 ms. Time scale can be referred to the AH interval.

Intracellular APs recorded in different anatomic regions (see Table) were characterized into 3 groups as atrial, transitional, and AV nodal cells, consistent with the results of previous studies. All APs obtained from the atrium, FP, and SP zone 1 did not have significant differences (P>0.05 in all groups), suggesting that similar types of atrial tissue surrounded KT. There were no significant differences (P>0.05 in all groups for all AP parameters) between transitional cells and SP zone 2. APs recorded in zone 3, particularly in deeper layers or near the edge of the tricuspid valve, had identical configurations (P>0.5 in all groups) compared with those recorded in the AV node, suggesting a posterior extension of the AV node and therefore an asymmetrical transitional zone surrounding the AV node. The SP was located in zones 2 and 3 and consisted of 2 types of cells, one being the transitional cell and the other being the AV nodal cell. From the FP to the transitional zone and AV node, AP amplitude, maximum diastolic potential, and maximum rate of rise at phase 0 (dV/dt) gradually decreased and resulted in a progressive delay in AV nodal conduction. The longest APD at 90% repolarization was found to be within the transitional zone rather than in the FP region.

Optical APs with double peaks were observed within the AV node or SP zone 3, consistent with a multilayer conduction pattern. Results from one of the experiments are shown in

### Results

#### Characterization of AV Nodal Conduction Properties

A picture of an AV nodal preparation is shown in Figure 1A. The FP was identified as the earliest retrograde atrial activation sites located near the anterior atrial septum outside Koch’s triangle (KT). The slow pathway (SP) region between the coronary sinus (CS) ostium and the tricuspid annulus was divided equally into 3 zones (Figure 1B). Optical APs recorded during atrial pacing at 800 ms are shown in Figure 1C. Representative optical and intracellular APs obtained from the atrium, FP, transitional zone, AV node, and SP are superimposed and displayed in Figure 1D. Each optical AP represents a voltage signal summated from many cells. The optical AP duration (APD) was expected to be longer than the intracellular APD as shown in Figure 1D, especially in the AV nodal and SP areas. Thus, repolarization times cannot be determined accurately from these optical APs and were measured from microelectrode recordings. In general, the configuration of the optical APs appeared to be similar to the intracellular APs except in areas with slow conduction, such as the AV node or SP.

#### Action Potential Parameters Obtained in Different Anatomic Regions

<table>
<thead>
<tr>
<th>Group</th>
<th>APA, mV</th>
<th>MDP, mV</th>
<th>APD90, ms</th>
<th>dV/dt, V/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrium (n=10)</td>
<td>90.5±1.1</td>
<td>−75.3±1.1</td>
<td>160.9±1.3</td>
<td>105.3±7.1</td>
</tr>
<tr>
<td>FP (n=12)</td>
<td>90.4±1.6</td>
<td>−74.8±1.4</td>
<td>163.1±2.9</td>
<td>109.3±5.0</td>
</tr>
<tr>
<td>SP-Z1 (n=6)</td>
<td>90.1±2.1</td>
<td>−74.2±1.6</td>
<td>165.4±3.1</td>
<td>104.6±8.3</td>
</tr>
<tr>
<td>Group 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (n=12)</td>
<td>80.0±0.8</td>
<td>−68.9±0.6</td>
<td>196.6±3.3</td>
<td>41.9±3.4</td>
</tr>
<tr>
<td>SP-Z2 (n=10)</td>
<td>78.9±1.0</td>
<td>−67.2±0.7</td>
<td>194.7±2.0</td>
<td>38.8±4.4</td>
</tr>
<tr>
<td>Group 3</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AV node (n=12)</td>
<td>68.4±1.4</td>
<td>−60.5±1.0</td>
<td>179.5±2.8</td>
<td>15.8±1.7</td>
</tr>
<tr>
<td>SP-Z3 (n=12)</td>
<td>68.8±1.5</td>
<td>−60.6±1.0</td>
<td>175.9±2.3</td>
<td>14.9±1.2</td>
</tr>
</tbody>
</table>

Data were obtained from 6 experiments (n=number of cells). Statistical analysis by ANOVA showed all P>0.05 (range, 0.12 to 0.98) within each group and all P<0.01 between groups. APA indicates AP amplitude; MDP, maximum diastolic potential; APD90, APD at 90% of repolarization; TC, transitional cell; SP-Z1, SP zone 1; SP-Z2, SP zone 2; and SP-Z3, SP zone 3.
The preparation was paced at 800 ms. APs with relatively early activation were recorded in the superficial layer of the AV nodal region and correlated with the first peak of the optical AP. As the microelectrode penetrated 3 to 5 cell layers deeper (determined by the numbers of cells recorded as the microelectrode advanced), AV nodal APs with relatively late activation were obtained. Similar phenomena were also observed in SP zone 3, except that in this case, only 2 to 3 layers of penetration were required. These findings indicated that the AV node and SP were covered with a thin layer of atrial tissue, leading to the development of an early component of atrial cell activation and a late component of AV nodal cell activation. The 2 components of activation seen in the optical APs could be further separated by a premature extrastimulus (A2=200 ms) as shown in Figure 2B. Optical APs and His bundle electrogram (H) obtained before and after phenol application obtained before and after application of phenol are shown in Figure 2C. The tissue was stimulated at 800 ms. A decrease in atrial electrogram amplitude in the His tracing indicated that phenol likely destroyed the surface tissue. As the result, the early activation component in the optical AP was significantly reduced after phenol application. Phenol significantly prolonged the stimulus-to-atrium interval from 19.3 ±2.3 to 28.3 ±2.0 ms (47%, n=3; P<0.05) but only slightly delayed the AH interval from 114.3 ±4.7 to 118.3 ±4.9 ms (4%, n=3; P<0.05), suggesting that phenol mainly delayed intra-atrial conduction. As the result, the shape of AVNCFs after phenol application did not differ from those before phenol application. After applying phenol, it was difficult to obtain good uniform signals from all sites. Therefore, we were unable to analyze the conduction sequence after phenol application.

Our data are consistent the conclusion reached by others that the double components of AP were generated by the summation of asynchronously arriving wavefronts from the different layers. Two different optical activation maps have been constructed by using the maximum dV/dt to detect the activation time from the first or the second components. In the present study, however, we selected the APA-max as the activation time to construct a single optical map. Justifications for adapting this method are described extensively in the online data supplement.

To further confirm the presence of atrial tissue covering the AV nodal area, phenol was applied to the AV nodal surface to destroy the superficial (≈300 μm) layers. Optical APs obtained before and after application of phenol are shown in Figure 2C. The tissue was stimulated at 800 ms. A decrease in atrial electrogram amplitude in the His tracing indicated that phenol likely destroyed the surface tissue. As the result, the early activation component in the optical AP was significantly reduced after phenol application. Phenol significantly prolonged the stimulus-to-atrium interval from 19.3 ±2.3 to 28.3 ±2.0 ms (47%, n=3; P<0.05) but only slightly delayed the AH interval from 114.3 ±4.7 to 118.3 ±4.9 ms (4%, n=3; P<0.05), suggesting that phenol mainly delayed intra-atrial conduction. As the result, the shape of AVNCFs after phenol application did not differ from those before phenol application. After applying phenol, it was difficult to obtain good uniform signals from all sites. Therefore, we were unable to analyze the conduction sequence after phenol application.

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A typical activation map is shown in Figure 3C. The impulse appeared to be conducted rapidly over the atrial tissue, and it spread slowly toward the AV node through the transitional zone. As seen in intracellular recordings, conduction delay began in the transi-
Characterization of AV Nodal Reentry: Slow/Fast Type

AV nodal EBs were induced by atrial programmed stimulation in 12 preparations (slow/fast, n=8; fast/slow, n=2; and slow/slow, n=2). Among these, only one exhibited sustained reentrant tachycardia (slow/fast) of >15 ms’ duration.

Results obtained from one of the experiments with a slow/fast EB are summarized in Figure 4. The AVNFC (Figure 4A) demonstrated a jump at a coupling interval of 190 ms, suggesting dual AV nodal physiology. Activation maps of A2 obtained at A1A2 intervals of 350, 200, and 190 ms are shown in Figure 4B, C, and D, respectively. The activation sequence of A1 at a basic pacing cycle length of 800 ms (map not shown) was similar to that shown in Figure 3C with multiple AV nodal inputs. The activation patterns of A2 at A1A2 intervals of 210 to 400 ms were similar to the map shown in Figure 4B at 350 ms. As the coupling interval decreased, conduction over the SP gradually slowed. At a further decrease in the coupling interval from 200 ms to 190 ms, the impulse reached the refractoriness of the transitional cells, and therefore unidirectional block occurred at the junction between the FP and the AV node. Anterograde conduction then shifted from the FP to the SP, resulting in an abrupt increase (jump) in the AH interval and the initiation of a slow/fast type of EB. The corresponding optical APs and EB at an A1A2 interval of 190 ms are illustrated in Figure 4E and F. The earliest retrograde atrial activation was recorded in the FP outside KT, consistent with a slow/fast type of AV nodal reentry. The reentrant circuit was complete as shown in Figure 4F. After retrograde atrial activation, the impulse entered the SP and AV node again with similar sequence but no exit. The locations of conduction block and earliest retrograde atrial activation were further identified by optical movies.

Mapping data from the slow/fast EB indicate anterograde conduction block at the junction between the FP and the AV node, likely caused by the longest APD and refractory periods present within the transitional cells near the AV node. Initial conduction delay within the transitional cells allowed the SP sufficient recovery time, and delay in SP conduction permitted the AV node and FP enough recovery time to be activated again. Anterograde activation from the AV node to the His bundle and retrograde conduction from the AV node to the FP initiated the reentrant EB, which reentered the SP and terminated in the AV node. Atrial tissue located between the FP and SP was part of the reentrant circuit, suggesting that there was no upper common pathway.

Using the criterion of an A2H2 interval duration >50 ms for a 10-ms decrease in the A1A2 coupling interval, the abrupt jump in the AV node functional curve was observed only in 2 of the 8 experiments with slow/fast type of EBs. In the other 6 preparations, EBs with similar slow/fast reentrant circuits were induced with no clear jump. The AV nodal curve from one of these experiments is shown in Figure 5A.
maps at coupling intervals of 300, 220, and 210 ms are shown in Figure 5B, C, and D, respectively. The activation sequence of the EB induced at the coupling interval of 210 ms was consistent with the slow/fast type of reentry. However, anterograde conduction did not shift abruptly from the FP to the SP but moved gradually from the FP to the IP (Figure 4C). With a further decrease in the A1A2 coupling interval from 220 to 210 ms, anterograde conduction further shifted from the IP to the SP with an EB but no jump. The IP served as a bridge to smoothly transfer anterograde conduction from the FP to the SP during decremental atrial stimulation, resulting in a smooth AVNFC.

In 2 preparations, discontinuous AVNFCs were observed without an inducible EB. Anterograde conduction shifted from the FP to the SP but failed to propagate retrogradely to the FP.

Characterization of AV Nodal Reentry: Fast/Slow Type

Fast/slow AV nodal reentry was seen in only 2 of 12 preparations that had inducible EBs. The activation maps obtained from both experiments at a pacing cycle length of 800 ms were similar to that shown in Figure 3F with delayed SP activation in zone 3. Results obtained from one of the experiments are shown in Figure 6. The AVNFC and selected activation maps are shown in Figure 6A, B, C, and D. Corresponding optical APs recorded during EB are shown in Figure 6E and F. As the pacing coupling interval decreased, delayed conduction in the AV node and FP became more obvious, as shown in Figure 6B and C. At a further decrease in A1A2 coupling interval to 220 ms, anterograde conduction markedly delayed within the AV node, resulting in prolongation of A2H2 to 576 ms. Meanwhile, retrograde conduction slowly propagated through the SP, allowing the atrial tissue located near the CS ostium to be activated again as an exit site. The AV node anterogradely conducted the subsequent EB at a relatively fast speed with a short AH interval, creating the fast/slow type of AV nodal reentry. Again, no upper common pathway was present because atrial tissue located between the FP and SP participated in the reentrant circuit.

As shown in Figure 6A, a classic jump was observed in the AVNFC at an A1A2 coupling interval of 230 ms. The A2H2 interval abruptly increased by 261 ms (from 335 ms at A1A2 interval of 240 ms to 596 ms at A1A2 interval of 230 ms). However, anterograde conduction between coupling intervals of 240 to 220 ms took the same pathway as shown in the corresponding activation maps in Figure 6C and D, suggesting that the jump was caused by conduction delay within the compact AV node rather than by a shifting of the conduction over another pathway. A similar jump was also seen in the other preparation with fast/slow reentry.

Characterization of AV Nodal Reentry: Slow/Slow Type

Slow/slow AV nodal EBs were induced in 2 experiments. Both preparations exhibited delayed activation in the SP,
similar to the activation map shown in Figure 3F. Results obtained from one of the experiments are summarized in Figure 7. AVNFC and activation maps of A2 at coupling intervals of 300, 220, and 190 ms are shown in Figure 7A, B, C, and D, respectively. Optical APs and His bundle electrogram recorded at 190 ms with an EB are displayed in Figure 7E and F. As the coupling interval decreased, anterograde conduction gradually shifted from the FP to the IP, while retrograde conduction propagated slowly over the SP without exit (Figure 7C). With a further decrease in the coupling interval to 190 ms, both anterograde and retrograde conduction maintained the same pathway, but additional conduction delay in the SP allowed the adjacent atrial tissue to recover and therefore to be activated again as an exit site, initiating the slow/slow AV nodal reentrant beat. The propagation shifting from the FP to the IP created a smooth AVNFC (Figure 7A). The slow/slow AV nodal reentrant circuit was characterized as anterograde conduction over the IP and retrograde conduction over the SP, with the earliest atrial activation located near the CS ostium. Like the other 2 types of AV nodal reentrant EBs, no upper common pathway existed.

In another experiment, similar slow/slow EBs and sustained tachycardia were induced. The reentrant tachycardia lasted >15 minutes and was terminated by burst rapid atrial pacing. The reentrant circuits and activation sequences of the EBs and tachycardia were identical to a constant tachycardia cycle length (see additional data in the online data supplement).

At the end of the experiments, the SP was selectively interrupted by a surgical incision (slow/fast, n=4; fast/slow, n=2; and slow/slow, n=2). AV nodal reentrant EBs were no longer induced in all preparations.

**Discussion**

**Major Observations**

**Three Preferential AV Nodal Input Pathways**

Our data suggest that 3 preferential AV nodal input pathways exist in all normal canine hearts regardless of the presence or absence of AV nodal EBs. These pathways represent atrio-nodal connections outside the AV node rather than longitudinal dissociation within the AV node. At a short coupling interval, unidirectional block occurs, and these functional pathways can separate into discrete reentrant pathways for anterograde and retrograde conduction. These observations are consistent with more recent data suggesting that multiple AV nodal input pathways might exist because anterograde AV conduction remains after ablation of both the FP and the SP.

**Absence of Upper Common Pathway**

The reentrant circuits for the slow/fast (counterclockwise) and fast/slow (clockwise) EBs are consistent with the previous hypothesis and identical to those seen in patients with similar types of AVNRT. The reentrant circuit for the slow/slow EB is different from that previously proposed. However, anterograde conduction actually occurs over the IP and retrograde conduction over the SP rather than over 2 separate SPs. Despite different reentrant circuits, atrial tissue surrounding KT is clearly involved in all 3 types of AV nodal reentry and suggests no upper common pathway. It is difficult to compare our results to those of a previous study under different experimental conditions. Taking into account the AV nodal blood supply, it is likely that the AV node was not totally isolated from atrial tissue in that study; otherwise, it would have become ischemic or infarcted in the setting of a completely dissected KT. Thus, reentry still could have involved atrial tissue. Recent optical mapping data obtained from isolated rabbit AV nodal preparations also suggest that functional AV nodal pathways are located outside the compact AV node and that AV nodal reentry involves atrial and transitional cells.

**Unidirectional Block in the Transitional Zone**

It is generally believed that in the slow/fast type of AVNRT, unidirectional block occurs within the FP because of its longer refractory period. However, our data indicate that the longest APD is located in the transitional cells, where slow conduction and unidirectional block occurred during a normal heart rate and AVNRT. The combination of an asymmetrical transitional zone around the AV node with its posterior extension and 3 preferential atrio-nodal pathways provide a unique model for the development of multiple types of AVNRT.
No Direct Relationship Between AV Nodal Jump and AV Nodal Input Pathways

Our mapping data indicate the following: (1) An abrupt jump in the AH interval resulted either from shifting of anterograde conduction from the FP to the SP or from abrupt conduction delay within the AV node through the same FP. (2) Anterograde conduction over the IP smoothed the transition from the FP to the SP, producing AVNRT with a continuous AVNFC. (3) Similarly, anterograde conduction over the SP without retrograde exit can cause a discontinuous AV nodal curve with no inducible AVNRT.

AV Nodal Posterior Extension

Previous studies have provided evidence to support the concept of a posterior AV nodal extension and suggested that the posterior AV nodal extension was involved in slow pathway conduction. Previous studies have provided evidence to support the concept of a posterior AV nodal extension and suggested that the posterior AV nodal extension was involved in slow pathway conduction. Our data are compatible with this conclusion and further indicate that the slow pathway existed in all normal canine hearts and that both transitional cells and posterior extension of AV nodal cells likely comprise the cellular substrate of this structure.

Limitations of the Present Study

Optical mapping is technically limited in that it cannot reconstruct the 3-dimensional structure of the AV node. The APA-max method also has several limitations. Questions related to these limitations are discussed further in the online data supplement.

Clinical Implications

The new observations in the present study have several clinical implications. First, the finding that the SP is involved in all 3 types of reentrant circuit explains the mechanism of cure by SP ablation applied in patients with various types of AVNRT. Second, multiple retrograde atrial exit sites will be expected in atypical AVNRT because retrograde activation conducts from the AV node to the posterior extension region, where multiple connections exist between the SP and atrial tissue near the CS. Finally, the FP is not involved in the reentrant circuit of slow/slow EBs, consistent with previous findings that FP ablation is not effective in some patients with atypical AVNRT.

Acknowledgments

Dr Jianyi Wu was supported in part by a Fellowship Award from the North American Society of Pacing and Electrophysiology. This research was also supported in part by grant 9930347Z from American Heart Association. We appreciate the generous help and advice of Dr Igor R. Efimov during the construction of our optical mapping system.

References

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Circ Res. 2001;88:1189-1195; originally published online May 24, 2001; doi: 10.1161/hh1101.092187

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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Mechanisms Underlying the Reentrant Circuit of AV Nodal Reentrant Tachycardia

In Isolated Canine AV Nodal Preparation Using Optical Mapping

Jianyi Wu, MD, Jiashin Wu, PhD, Jeffrey Olgin, MD, John M. Miller, MD, and Douglas P. Zipes, MD

Expanded Material and Methods

Additional data on AV nodal preparation

The preparation was perfused with Tyrode’s solution (in mmol/L, NaCl 128.0, KCl 4.7, NaHCO3 20.1, NaH2PO4 0.4, MgSO4 1.2, CaCl2 2.2, glucose 11, bubbled with a 95% O2 and 5% CO2 gas mixture). Within 2 min of establishing perfusion, the preparations spontaneously developed a junctional rhythm at cycle length of 1200-2500 ms. Preparations were then stimulated at basic cycle length of 800 ms. Data acquisition began after >30 min stabilization period. At the end of the experiments, the preparations were fixed in 10% formalin solution. The AV nodal thickness measured under the microscope was 109±20 µm (from 85 to 135 µm, n=10).

Intracellular microelectrode recording of APs

Glass microelectrodes had resistances of 10-15 MΩ when filled with 3 M KCL solution. A microelectrode preamplifier (Model 8700, Dagan Corp., Minneapolis, MN) was used to record intracellular APs. Data were filtered at 1.0 kHz and digitalized at 50 kHz. AP parameters
measured included AP amplitude, maximum diastolic potential, APD at 90% repolarization (APD90) and peak rate of rise of depolarization (dV/dt) in V/sec.

**Additional Figures and Supporting Information**

*Justification of the new method (APA-max) used to determine the activation time*

Based on the cell dimension and the depth of optical mapping area, the total number of cells in each optical mapping site were estimated to be up to 20,000. In the atrial and fast pathway region, atrial cells within each mapping site had similar configurations, with rapid upstroke of depolarization and were activated within a very short time. Optical action potentials obtained from these regions had one component of activation. Consequently, both the dV/dt-max and the APA-max of optical action potential correlated well with intracellular action potential recorded from any one of the cells. Both the dV/dt-max and APA-max were likely to slightly over- or underestimate the activation time as described latter. However, in the AV node, transitional zone and slow pathway regions, atrial and AV nodal cells overlapped each other and had different types of action potential configurations. As a result, optical action potentials recorded from these regions had two components of activation. The dV/dt-max of the first component could be detected and represented the activation of superficial atrial cells (see Online Figure 1), whereas, the dV/dt-max of the second component was difficult to detect objectively because the second component was usually exhibited as a slow depolarizing curve (Online Figure 1A), likely due to slow activation between AV nodal cells. For that reason, the conventional method of dV/dt-max could not be used to accurately detect the optical activation time in regions where atrial and nodal cells overlapped.
In contrast, APA-max represented the time of maximum number of cells depolarized within the mapping site. It could be objectively detected by the computer and used as an alternative method to determine the activation time. As a matter of fact, APA-max had been used as one of the methods to detect the activation and show the activation sequence in the optical mapping movies. The activation sequences displayed by the movies were very consistent and independent of any subjective measurements. Therefore, we adapted the APA-max method to detect the activation time to construct the optical maps. The advantage of using this method was that it eliminated the interference of the superficial atrial tissue and created a single map to show a continuous activation sequence as seen in optical activation movies. The disadvantage was that it underestimated the activation time by a delay of 15 ms as shown in Online Figure 1A, likely due to delayed activation between cells within each mapping site and the "spike-and-dome" morphology of atrial action potentials. However, this delay was unlikely to significantly alter the activation sequence because the delayed times were expected to be similar in each site by using the same method of APA-max. On the other hand, the dV/dt-max might overestimate the activation time with earlier detection because cells within the mapping site were not activated simultaneously, as evidenced by the fact that the optical action potential durations were longer than that recorded by microelectrode. The limitation of dV/dt-max became significant in regions with overlapping different types of cells. As shown in Figures 1D and 2B, the APA-max generally correlated with the peak of intracellular action potential in different anatomic regions, suggesting that APA-max was a validated alternative method to determine the activation time of the optical action potentials.
The second derivative provided additional evidence to support the hypothesis of multi-layer conduction. It was not intended to introduce a new method to determine the activation time because the two components were difficult to separate during baseline pacing or within the transitional zone (see text of the original manuscript).

*Interference of double peaks of action potentials in determining the activation time*

The double peaks of optical action potentials resulted from the overlap of atrial and AV nodal cells in the AV node, slow pathway and transitional zone. In previous studies, two separate optical activation maps were constructed by using dV/dt-max to detect the activation time from the first and second peaks, respectively. Obviously, maps created by this method using the dV/dt-max also had the similar problems of ignoring the other components. Therefore, the interference of double peaks of action potentials in determining the activation time always existed regardless of which method of dV/dt-max or APA-max was selected. Since optical action potentials obtained from the atrium and fast pathway have one component of activation, this concern is likely not applied to these regions. Data from both previous and present studies consistently suggested that the first peak represented the activation of the superficial atrial tissue and the second peak represented the activation of AV nodal cells located at the deeper layers. Accordingly, the information (first peak) ignored by using the method of APA-max was the activation of the superficial atrial tissue and was relatively irrelevant regarding AV nodal conduction. In fact, the single map created by using the APA-max actually limited the interference of superficial atrial tissue and represented AV nodal activation sequence as seen in
the optical movies. As shown in Online Figure 2B, the activation sequence of the first component represented the activation of atrial tissue.

*Delay of activation time measured by using the method of APA-max*

Using the method of APA-max, the activation times in the atrial area were delayed by ~15 ms as compared to that measured by the dV/dt-max (Online Figure 1A). This delayed time actually reduced the artifact related to the line of block or slow conduction at the transitional zone where the two components started to merge. This delay was unlikely to significantly alter the activation sequence because all mapping sites were measured by the same method with a similar amount of time shift. However, this time shift underestimated the activation time in the AV nodal region and created an unusual phenomenon in that the activation in the AV nodal region was slightly after the His bundle as shown in Online Figure 2. On the other hand, the activation time measured by the conventional method of dV/dt-max was 43 ms, which was much earlier than the His bundle electrogram and correlated with the activation of the atrial electrogram. In comparison, APA-max appeared to be an alternative optimal method to detect the activation time in the AV nodal region.

*Consistency of the activation pattern during sustained AV nodal reentrant tachycardia*

As shown in Online Figure 3, the slow/slow reentrant circuits during sustained reentry were very stable, as indicated by the tachycardia cycle length and the AH intervals. The activation sequences during tachycardia were similar to that shown in Figure 4D. The noise of the optical recording appeared to be low and did not interfere with the signal analysis, indicating that
averaging optical tracings will likely not provide additional information. The reentrant circuits for the echo beat and tachycardia were identical.

Effects of Cytochalasin D

We have recently demonstrated that cytochalasin D could limit tissue contraction with no effect on APD in isolated canine ventricular myocytes or on conduction velocity in canine ventricular tissue preparations.\textsuperscript{1,2} APs recorded from six atrial cells in two preparations showed no significant differences between control conditions and after exposure to cytochalasin D, in AP amplitude (90.4±1.2 vs. 90.5±1.3 mV, p=0.714), maximum diastolic potential (-75.1±1.2 vs. -74.6±1.4 mV, p=0.545), APD90 (155.4±1.4 vs. 156.3±1.5 ms, p=0.126) and dV/dt (184.5±14.5 vs. 178.6±16.7 V/s, p=0.114). AH intervals obtained at a pacing cycle length of 800 ms showed no significant change after cytochalasin D (115±4 ms before vs. 117±5 ms after, n=6, p=0.112), suggesting that cytochalasin D had no effect on AV nodal conduction

Other Supporting Information: Limitations of the present study

Optical mapping in three-dimensional AV nodal structure

The optical mapping is technically limited because one can not reconstruct the three-dimensional structure of the AV node. Several questions relate to this limitation. First, we considered the relationship between the depth of optical mapping and the thickness of AV node. The tissue thickness measured at the compact AV nodal region was ~109 µm, whereas, the depth of the optical field was likely limited to 500-600 µm. Because the AV node is an elliptical structure,\textsuperscript{3} the largest two dimensional cross-sections at the center of AV node are located within
the plane of ~550 µm below the surface of endocardium, suggesting that most of the AV nodal cells were located within the depth of optical mapping field. Second, we asked how activation wavefronts entered the AV node. Based on microelectrode recordings, the activation time in the superficial layers of the AV node was earlier than that in the deeper layers, indicating that anterograde conduction entered the AV node from the superficial layer to the deeper layer. Third, we questioned where anterograde conduction block occurred. Since activation conducted from superficial to deeper layers, it would be very unlikely that block of the wavefronts only occurred on the surface and the wavefronts entered into the node at a deeper layer. Alternatively, conduction delay or block occurred in the transitional zone and was associated with progressive decreases in action potential amplitude, maximum diastolic potential and dV/dt as shown in Table 1.

*Limitations of using APA-max to determine the activation time*

There were several limitations when using the method of APA-max to detect the activation time to construct an optical map. These included an underestimation of the activation time by ~15 ms, due to delayed activation between cells within the mapping site and/or the "spike-and-dome" morphology of atrial action potential configuration; failure to consider the activation of superficial atrial cells but that limitation simplified mapping the pattern of multi-layer conduction; and potential influence of low-frequency noise or artifact that required further justification by interpolation.

*Low common pathway and left side variant of AVNRT*
Retrograde VA conduction during ventricular pacing was not present because most of the ventricular tissue was cut off and the remaining ventricular tissue was not perfused through the first septal perforator coronary artery branch. Therefore, the low common pathway was not evaluated. Another limitation is that the left side variant of AVNRT was not seen in this study, likely due to the fact that the left atrium was trimmed off.

References
Online Figures Legends

Online Figure 1: Determination of activation time using conventional method of dV/dt-max and new method of APA-max. Data shown here were obtained from the same experiment shown in Figure 3A, B and C in the manuscript. A: Representative optical action potentials obtained from atrium (A), fast pathway (FP), AV node (AVN) and slow pathway (SP) zone 2. The vertical lines indicate the activation time as determined by the dV/dr-max and APA-max, respectively. The dV/dt-max accurately estimated the activation time in atrium and FP regions without delay but could not be used in the AV nodal and SP area due to a slow depolarizing curve, whereas the APA-max is an alternative method to determine the activation time with ~15 ms delay. B: Activation map obtained by dV/dt-max, which represented the activation sequence of the superficial atrial tissue. The corresponding activation map using APA-max is shown in Figure 3; panel C in the manuscript. (See text for further justification).

Online Figure 2: Delayed activation times obtained by APA-max compared with using dV/dt-max. AV nodal optical action potential and His bundle electrogram are superimposed and displayed at fast sweep speed. Data were obtained from the same tracings shown in Figure 4E in the manuscript. The vertical lines indicate the activation times obtained in computer by dV/dt-max and APA-max. The second component of activation exhibited as a slow rising curve and the activation time could not be detected by the dV/dt-max. The activation time of the first component obtained by dV/dt was 43 ms and correlated with the atrial electrogram, whereas, the
APA-max was 12 ms after the His bundle electrogram, likely due to an underestimation of the activation time by the APA-max, with delayed activation between AV nodal cells.

Online Figure 3: Optical action potentials obtained from atrium (A), intermediate pathway (IP), AV node (AVN) and atrial tissue near the slow pathway (SP) are displayed together with His bundle electrogram recorded during sustained AVNRT (slow/slow form). The reentrant circuits were very stable as indicated by the tachycardia cycle length (475 ms) and AH interval (305 ms). The activation sequences were similar to that shown in Figure 7D. The site (star) of earliest retrograde atrial activation was recorded at the atrial tissue near the slow pathway (SP). Anterograde and retrograde conduction was over the intermediate (IP) and slow pathways, respectively. The signal-to-noise ratio was relatively low, suggesting that averaging data will likely not provide additional information.
Online Figure 2

A - 30 ms
His - 135 ms
AH = 105 ms

APA-max (147 ms)
DV/dt-max (43 ms)