Computer Three-Dimensional Reconstruction of the Atrioventricular Node

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Abstract—Because of its complexity, the atrioventricular node (AVN), remains 1 of the least understood regions of the heart. The aim of the study was to construct a detailed anatomic model of the AVN and relate it to AVN function. The electric activity of a rabbit AVN preparation was imaged using voltage-dependent dye. The preparation was then fixed and sectioned. Sixty-five sections at 60- to 340-μm intervals were stained for histology and immunolabeled for neurofilament (marker of nodal tissue) and connexin43 (gap junction protein). This revealed multiple structures within and around the AVN, including transitional tissue, inferior nodal extension, penetrating bundle, His bundle, atrial and ventricular muscle, central fibrous body, tendon of Todaro, and valves. A 3D anatomically detailed mathematical model (≈13 million element array) of the AVN and surrounding atrium and ventricle, incorporating all cell types, was constructed. Comparison of the model with electric activity recorded in experiments suggests that the inferior nodal extension forms the slow pathway, whereas the transitional tissue forms the fast pathway into the AVN. In addition, it suggests the pacemaker activity of the atrioventricular junction originates in the inferior nodal extension. Computer simulation of the propagation of the action potential through the anatomic model shows how, because of the complex structure of the AVN, reentry (slow–fast and fast–slow) can occur. In summary, a mathematical model of the anatomy of the AVN has been generated that allows AVN conduction to be explored. (Circ Res. 2008;102:975-985.)

Key Words: atrioventricular node ■ slow pathway ■ fast pathway ■ reentry ■ modeling

The AVN lies within the triangle of Koch bounded by the coronary sinus, tendon of Todaro, and tricuspid valve (Figure 1A). The function of the AVN is to conduct action potentials at an appropriate conduction velocity from the atria to the ventricles. Functionally, the AVN is complex. For example, the AVN shows dual pathway conduction: the slow pathway into the AVN runs from the isthmus (between coronary sinus and tricuspid valve) to the apex of the triangle of Koch, whereas the fast pathway is more cranial (Figure 1B). The AVN is a subsidiary pacemaker, and the leading pacemaker site has been reported to be within the slow pathway.1 AVN reentrant tachycardia is the most common reentrant arrhythmia in adults2; prevention involves ablation of the isthmus of Koch, whereas the fast pathway is more cranial (Figure 1A). The function of the AVN is to conduct action potentials (ie, anatomy). To understand the structure–function relationships of the AVN, the aim of the present study was to generate an anatomic model of the AVN and relate it to function. For research and teaching, there is an effort to build a “virtual heart.”3 This requires anatomic models in the form of mathematical arrays or finite element models for each part of the heart. Such models exist for the atria, ventricles, and sinoatrial node (SAN).3,4 Here is the first such anatomic model (mathematical array) of the AVN. The study was carried out on the rabbit, because the rabbit AVN preparation is amenable to experimentation and is widely used, and anatomic models already exist for the SAN, atria, and ventricles of rabbit.3

Materials and Methods

The heart was removed from New Zealand White rabbits, and the region of the AVN (boxed region in Figure 1A) was dissected. All of the work described here was carried out on the AVN preparation shown in Figure 1B. The preparation was stained with the voltage-sensitive dye di-4-ANEPPS [3-(4-(2-(6-(dibutylamino)-2-naphthyl)-trans-ethenyl)pyridinium)propanesulfonate], and electric activity was recorded, as previously described,3 during AVN pacemaking and reentry. Following recording, the preparation was fixed, paraffin-embedded, and sectioned in the plane shown by the vertical lines in Figure 1B (ie, in dorsal–ventral plane, roughly perpendicular to septal leaflet of tricuspid valve). Sections at 65 levels at 60- to...
340-μm intervals were stained with Masson’s trichrome to show histology. Sections adjacent to these were labeled using immunoenzyme for middle (160/165-kDa) neurofilament and connexin (Cx)43 to identify different cell groups. Neurofilament was used as a marker for conduction system tissue; neurofilament is a neuronal cytoskeletal protein that in the rabbit is exclusively expressed in the atrial and ventricular conduction system (it is not expressed in the working myocardium) and has previously been used as a marker of rabbit SAN and AVN myocytes, as well as ventricular conduction system.1,4 Connexins are responsible for electric coupling between heart cells, and Cx43 is the most abundantly expressed connexin isoform in the heart. Here, sections from just 5 levels (inferior nodal extension [INE] [start, middle, end]; penetrating bundle; and His bundle) are shown to illustrate the major features, but sections from all 65 levels were used to construct a 3D computer model (mathematical array) of the anatomy of the AVN. Firstly, the sections were outlined, divided into different cell types, and aligned based on the positions of the tract of nodal tissue and tendon of Todaro. Secondly, based on the interval between sections, a common coordinate system was used to define all sections. Thirdly, the vertical position of sections was adjusted so that the bottom edge of the model mapped onto the bottom edge of the original preparation. Finally, the voxel size of the array was adjusted to be 10×20×50 μm. Propagation of the action potential through the anatomic model was calculated using the cellular automaton or monodomain models. Although it was only feasible to generate an anatomic model for 1 preparation, we have confirmed the electrophysiological findings previously,1,5,6 and the anatomic findings were confirmed in more than 7 preparations. For further details of the methods used, see the online data supplement, available at http://circres.ahajournals.org.

Results
At the atrioventricular junction, the tract of neurofilament-expressing nodal tissue is divided into 2: the INE and the penetrating bundle. The INE is located in the right atrium and is continuous with the penetrating bundle. The penetrating bundle penetrates the fibrous tissue separating the atria and ventricles; it emerges in the ventricles as the bundle of His.

Inferior Nodal Extension
Figure 1C shows a Masson’s trichrome–stained section toward the start of the INE (left) and the adjacent neurofilament-labeled section (right; corresponding to region shown). D, Masson’s trichrome–stained section near middle of INE (left) and the adjacent neurofilament-labeled section (right; corresponding to region shown). Boxes a through d show location of images in Figure 1A through 2D. AM indicates atrial muscle; Ao, aorta; AoV, aortic valve; CFB, central fibrous body; CS, coronary sinus; CT, crista terminalis; IVC, inferior vena cava; LA, left atrium; LV, left ventricle; MV, mitral valve; PA, pulmonary artery; PV, pulmonary vein; RA, right atrium; RV, right ventricle; SVC, superior vena cava; tT, tendon of Todaro; TT, transitional tissue; TV, tricuspid valve; VM, ventricular muscle.
the middle of the INE. The position of the sections is given in millimeters and is shown in Figure 1B. In Figure 1C and 1D, the myocytes are stained purple, and connective tissue is stained blue. The atrial and ventricular tissues are separated by fibrous tissue (stained blue; Figure 1C and 1D). Other landmarks such as the tendon of Todaro can be seen (Figure 1D). Neurofilament labeling of the boxed regions in Figure 1C and 1D is also shown. The nodal tissue of the INE can be recognized because it expresses neurofilament (brown label), whereas the surrounding tissue does not (Figure 1C and 1D, right images). At the middle of the INE (Figure 1D), there is a vein that divides the myocytes of the INE (confirmed in more than 7 hearts). The nodal tissue lying above the vein appears to be continuous with atrial tissue, but the nodal tissue lying below may be isolated by the vein. (Of course, it is also isolated from ventricular muscle by fibrous tissue.) In contrast, toward the start of the INE, the vein is not present and the nodal and atrial tissues appear to be in direct contact (Figure 1C).

Figure 2A through 2D shows high magnification images of the Masson’s trichrome–stained section (left) as well as Cx43 labeling (right) in the boxed regions (labeled a through d) in Figure 1D. The myocytes of the INE are small and dispersed among connective tissue and do not express Cx43 (Figure 2D). The ventricular myocytes lying below the INE are large and densely packed (separated by relatively little connective tissue) and do express Cx43 (Figure 2C). At the middle of the INE, on the side of the aorta, there is a bundle of densely packed atrial muscle (Figure 1D, asterisk); atrial myocytes, like ventricular myocytes, are large and densely packed (separated by relatively little connective tissue), and express Cx43 (Figure 2B). However, at the middle of the INE, the majority of the tissue lying above the INE is loosely packed (Figure 1D). Here, we refer to this as “transitional tissue” because, although it does not express neurofilament (Figure 1D) and it does express Cx43 (Figure 2A) like atrial muscle, the myocytes are small and dispersed among connective tissue like nodal myocytes. Interestingly, above the start of the INE, there is no transitional tissue, only atrial muscle (Figure 1C). Figure 2I shows the diameter of the different myocyte types: myocyte diameter is significantly greater for the atrial and ventricular muscle; myocyte diameter is not significantly different between the transitional tissue and INE.

Figure 2F and 2I, whereas the lower nodal myocytes, although still small and dispersed, are Cx43-positive (Figure 2F and 2I).

Penetrating Bundle
Figure 3B shows a Masson’s trichrome–stained section at the start of the penetrating bundle. The nodal tissue (identified by neurofilament labeling; Figure 3B) is separated from transitional tissue above (as well as ventricular muscle below) by connective tissue: the nodal tissue is, therefore, enclosed. At this level, once again, there is an isolated bundle of densely packed atrial muscle on the side of the aorta (Figure 3B, asterisk). High-magnification images of the Masson’s trichrome–stained section, as well as Cx43 labeling in the boxed regions in Figure 3B, are shown in Figure 2G and 2H. Figure 2G shows images of the upper part of the penetrating bundle: the nodal myocytes are small (Figure 2I) and densely packed and show weak and punctuate Cx43 labeling. We refer to this region as the “compact node.” Figure 2H shows images of the lower part of the penetrating bundle: the lower nodal myocytes are small (Figure 2I) and dispersed among connective tissue and show stronger Cx43 labeling; they are continuous, with the lower nodal myocytes at the end of the INE discussed above.

His Bundle
Figure 3C shows a Masson’s trichrome–stained section at the level of the His bundle at the point where it divides to form the left and right bundle branches. The nodal tissue (identified by neurofilament labeling) is separated from the atrial muscle above by the central fibrous body and from the ventricular muscle below by connective tissue (Figure 3C). Therefore, at this point, the tract of nodal tissue has emerged into the ventricles. The myocytes of the His bundle are small (Figure 2I), neurofilament-positive, and Cx43-positive (data not shown).

Nervous Innervation
Prominent nerve trunks were observed at the AVN. The inset in Figure 3B (***) shows 2 nerve trunks located next to a vein near the penetrating bundle. Within the nerve trunks, there is an abundance of nuclei (presumably belonging to supporting Schwann cells; Figure 3B). Another example is shown in the online data supplement.

Model of the AVN
In summary, at the atrioventricular junction, there is neurofilament-positive nodal tissue (loosely or densely packed and Cx43-negative or -positive), neurofilament-negative transitional tissue (loosely packed, Cx43-positive), and neurofilament-negative atrial and ventricular muscle (densely packed, Cx43-positive). Figure 4 summarizes the distribution of different cell types at all 65 levels studied: only the tract of nodal tissue and the nearby tissue is shown. Cx43-negative nodal tissue of the INE (red), compact node (bright yellow), Cx43-positive tissue of the penetrating bundle (purple), atrial muscle (peach), transitional tissue (green), ventricular muscle (pink), tendon of Todaro (dark blue), aortic valve (violet), connective tissue (light blue), and fatty tissue (light yellow) are shown in Figure 4. Additional views of the sections are shown in the online data supplement. The sections in Figure 4 were used to generate a 3D anatomic model of the AVN. The model is a mathematical array with ≥ 13 million elements and is available in the online data supplement.
Figure 2. High-magnification images of different myocyte types at atrioventricular conduction axis. A through H, transitional tissue (A), atrial muscle (B), ventricular muscle (C), middle of INE (D), upper nodal tissue of end of INE (E), lower nodal tissue of end of the INE (F), compact node at the start of the penetrating bundle (G), and lower nodal tissue at the start of the penetrating bundle (H). Sections stained with Masson’s trichrome are shown on the left, and adjacent Cx43-labeled sections are shown on the right. A through D were taken from regions a through d in Figure 1D; E and F are from regions e and f in Figure 3A; and G and H are from regions g and h in Figure 3B. I, Mean(±SEM; n=15) diameter of different myocyte types. Numbers 1 to 9 are significantly different (P<0.05; 1-way ANOVA) from the appropriately numbered region.
Various views of the isosurface model are shown in Figure 5 (the model as viewed from the right atrium/ventricle is shown at the top; and as viewed from the left atrium/ventricle/aorta, at the bottom). The model is either shown with all cell types (Figure 5A and 5B) or with some cell types removed (Figure 5C through 5F). Figure 5A shows that the INE is located between the coronary sinus and the tricuspid valve, the end of the INE is covered by transitional tissue, the penetrating bundle begins at the apex of the triangle of Koch (formed by coronary sinus, tendon of Todaro and tricuspid valve), and the penetrating bundle and His bundle are covered by connective tissue ("sheath" in Figure 5A). Figure 5C and 5D shows the model after removal of the transitional tissue and connective tissue. The boundary of the 3 types of nodal tissue (red, yellow, and purple) can be seen; note the tract of Cx43-positive lower nodal tissue (purple) projecting into the INE. The compact node (which weakly expresses Cx43; yellow) is located at the junction where the Cx43-negative nodal tissue (red) meets the Cx43-positive nodal tissue (purple). Figure 5D shows a prominent bundle of densely packed atrial muscle running along the nodal tissue (the bundle has been highlighted). This muscle bundle is the bundle discussed in Figures 1D and 3A and 3B (asterisk).

Figure 5E and 5F shows the nerve trunks (in white). One nerve trunk runs from the His bundle, past the penetrating bundle (as shown in Figure 3B) and along the tendon of Todaro. Another nerve trunk is next to the INE. The course of the vein first highlighted in Figure 1D is shown in Figure 5E and 5F (green). Movie 1 in the online data supplement is an animation of the model.

**Fiber Orientation**

Myocyte orientation was identified from the cross-sectional view of myocytes in the Masson’s trichrome–stained sections. Figure 6 shows myocyte orientation throughout a section (at level of line in Figure 6B and 6C) and on the surfaces of the AVN preparation. Whereas myocytes generally run horizontally in the upper atrial muscle, in the lower atrial muscle, transitional tissue, and nodal tracts, they generally run vertically.

**Correlation of Structure and Function: Activation Sequence of AVN**

In the absence of stimulation, the AVN shows pacemaking. In the same preparation as used to construct the model, di-4-ANEPPS was used to map the spread of the action
potential during spontaneous activity; this is shown as a color contour map in Figure 7A. The map has been superimposed on the anatomic model. This shows that the action potential was first initiated at the end of the INE at the junction of the upper and lower nodal tissues. From here, the action potential spread in both directions along the tract of nodal tissue. Later, the action potential propagated into the atrial tissue, but this is not shown. This is the same behavior as we have reported previously.

Again, in the same preparation as used to construct the model, the spread of the action potential during reentry was mapped. The preparation was stimulated at the His bundle; 2 stimuli (S1 and S2) were delivered. The action potential in response to the S1 stimulus exited into the atrial muscle via the fast pathway (data not shown). Figure 7B shows the response to the S2 stimulus; resultant action potentials recorded at 27 sites are shown; the position of the recording sites is shown in Figure 7D. The action potential propagated from the apex of the triangle of Koch to the isthmus between the coronary sinus and the tricuspid valve (sites 1 to 13; Figure 7B and 7D). The conduction velocity from sites 1 to 13 was low (2 to 13 cm/sec; Figure 7C): conduction was occurring along the slow pathway (the fast pathway was refractory because the S1–S2 interval was shorter than the refractory period of the fast pathway). At site 13, the action potential broke out into the atrial muscle. For comparison, Figure 7B shows atrial action potentials on a path parallel to the slow pathway (sites 14 to 27). Figure 7C shows that action potential conduction through the atrial muscle was faster (up to 69 cm/sec). Following excitation of the atrial muscle, there must have been anterograde conduction along the fast pathway (which was no longer refractory), resulting in a reentry beat (Figure 7B, asterisk), which propagated along the slow pathway and out into the atrial muscle once more. Extracel-
lular bipolar electrodes were used to record potentials from the high and low crista terminalis, interatrial septum, and His bundle, and the signals are shown at the top of Figure 7B (the recordings confirm that following the S2 stimulus, there were at least 2 action potentials: the action potential elicited by S2 stimulus and at least 1 reentry action potential). This is the same behavior as we have observed previously in 50% of preparations studied.6 The conduction pathway, followed by the action potential during reentry, is shown superimposed on the model of the AVN in Figure 7E; this suggests that the action potential first propagated along the INE (red) and then broke out into the atrial muscle at the isthmus. We know that there is possible contact between the atrial and nodal myocytes at this point (Figure 1C). Why did the action potential not break out earlier? As shown in Figures 1D and 5E, the prominent vein runs along this length of tissue and may electrically isolate the myocytes of the INE from transitional tissue/atrial muscle. The reentry shown in Figure 7B through 7E is known as fast–slow.

Correlation of Structure and Function: Simulation of Normal Conduction and Reentry

In the introduction of this report, we stated that the AVN owes its complexity of function to its complexity of structure. According to this hypothesis, it should be possible for the anatomic model to explain the complexity of function. To test this hypothesis, the anatomic model was used to calculate the activation sequence of the AVN. Action potential conduction was calculated using 2 different models. First, it was calculated using the simple cellular automaton model; although simple, the model is ideal for investigating the connectivity between the different tissues in the anatomic model. Secondly, results were confirmed using the monodomain model (including biophysically detailed models of the action potential). The anatomic model included a simplified version of myocyte orientation. Conduction was assumed to be faster parallel to the long axis of the myocyte than perpendicular to it: the anisotropic ratio was assumed to be 3:1. Conduction was also assumed to vary in the different tissues: for example, with the cellular automaton model, “coupling” in the atrial muscle/transitional tissue, INE, and penetrating bundle was assumed to be in the ratio 1:0.14:0.57. Further details of the simulations can be found in the online data supplement.

During spontaneous activity, the calculated activation sequence of the AVN (calculated using the cellular automaton or monodomain models) was similar to that seen in experiments (data not shown). The activation sequence of the AVN during normal anterograde conduction was similar regardless of whether it was calculated using the cellular automaton or monodomain models; Figure 8A shows the activation sequence as calculated using the monodomain model. The blue lines are isochrones at 5-ms
intervals. The atrial muscle was stimulated at the crista terminalis (top left). The action potential propagated through the atrial tissue at $\approx 28$ cm/sec (monodomain model) or $\approx 46$ cm/sec (cellular automaton model) (see the online data supplement for details), similar to that demonstrated in experiments (35±17 cm/sec$^3$). With both models, the action potential first entered the tract of nodal tissue at 2 points. The first point was at the end of the INE (next to the penetrating bundle) via the transitional tissue (green); we know that there is possible contact between the transitional and nodal tissues at this point (Figure 3A); this conduction pathway may correspond to the fast pathway route observed in experiments. Secondly, the action potential entered toward the start of the INE; this is the exit point identified during retrograde conduction (Figure 7E), and we know that there is possible contact between atrial muscle and nodal tissue at this point (Figure 1C); this conduction pathway may correspond to the slow pathway route observed in experiments. The action potential did not enter the tract of nodal tissue at other points because in the model, the nodal and atrial tissues are assumed to be isolated from each other by the prominent vein along this length of tissue (Figures 1D and 5E). The action potential propagated along the tract of nodal tissue at $\approx 7$ cm/sec (monodomain model) or 3 cm/sec (cellular automaton model) (see the online data supplement for details), similar to in experiments (2 to 10 cm/sec$^3$). From the 2 entry points, the action potential propagated both anterogradely and retrogradely along the INE; the action potentials met and annihilated each other (Figure 8A). The action potential reached the His bundle in $\approx 80$ to 85 ms with both models, and this is comparable to the conduction time in experiments ($\approx 96$ ms$^8$). The activation sequence is available as a movie (supplemental Movie 2). The right side of Figure 8A shows that, with the monodomain model, the calculated intracellular action potential waveforms 1 to 5 along the conduction pathway are similar to those recorded experimentally.$^8$

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**Figure 6.** Myocyte orientation at atrioventricular junction axis. A, Myocyte orientation in single section (from level of the vertical line in B and C). B and C, Myocyte orientation on surfaces of AVN preparation viewed from the right (B) or left (C) side of heart. Black circles indicate transversely cut myocytes; lines, longitudinally cut myocytes.
It was possible to simulate fast–slow reentry (Figure 7B through 7E) using the anatomic model of the AVN (cellular automaton model used). For reentry to occur, it is necessary that the INE (putative slow pathway) is electrically isolated from atrial tissue except at its 2 ends; as discussed above, this is the case in the model. In addition, it is necessary to produce unidirectional block: from experiments on the rabbit AVN, it is known that the refractory period of the fast pathway is longer than that of both the atrial muscle and slow pathway, and this property was introduced into the cellular automaton model (see the online data supplement for details). A simulation is shown in Figure 8B. The anatomic model was stimulated at the His bundle as in the experiment in Figure 7B through 7E; 2 stimuli (S1 and S2) were delivered. The S1 action potential exited into the atrial muscle via the transitional tissue (green), the putative fast pathway (Figure 8B). However, the premature S2 action potential failed to exit via the transitional tissue, because the S1–S2 interval was shorter than the refractory period of the transitional tissue. Instead, the S2 action potential exited into the atrial muscle via the INE (red; putative slow pathway) (Figure 8B) because the refractory period of the INE is shorter. The action potential then propagated anterogradely along the fast pathway (transitional tissue was no longer refractory) back into the tract of nodal tissue (Figure 8B). This resulted in reentry (Figure 8B and supplemental Movie 3). As shown in Figure 8C, in the model, the timing of the action potentials at different points on the reentry circuit is similar to that seen in experiments (Figure 7B, top).

There are 3 types of AVN reentry: slow–fast and intranodal, as well as fast–slow. Slow–fast reentry is clinically known as the “common type,” and using the anatomic model, it was also possible to simulate slow–fast reentry (see the online data supplement). In the model, as in the clinic,
Ablation of the isthmus abolished reentry, as dramatically shown by supplemental Movie 4.

Discussion

For the first time, based on histology and immunolabeling of marker proteins including neurofilament, which clearly and unambiguously identifies nodal tissue,1,4 we have generated a 3D anatomic model of the AVN, including 10 tissue types, as a mathematical array. Comparison of the model with electric activity measured in the same preparation shows the role of the different components of the AVN; for example, it demonstrates the importance of the INE in pacemaking, as well as atrioventricular nodal reentry. Furthermore, the anatomic model (together with simple or biophysically detailed models of action potential propagation) can be used to simulate the activation sequence of the AVN during normal anterograde conduction through the AVN, atrioventricular junctional pacemaking, and slow–fast and fast–slow atrioventricular nodal reentry.

To show the relationship between the model and other structures, Figure 8D shows the model of the AVN, together with our model of the SAN,4 superimposed on a 4-chamber view of the heart. The general anatomic features of the rabbit AVN described here are similar to those reported by other investigators, although different investigators may use different terminology for the various structures.9,10

Nature of Slow Pathway: Entry and Exit Points

Electrophysiologically, 2 pathways (slow and fast) have been described from the atrial muscle into the penetrating bundle (Figure 1B). Figure 7B through 7E shows retrograde conduction during reentry in the preparation used to construct the model: the action potential propagated retrogradely along the slow pathway and exited into the atrial muscle (fast pathway was refractory). Figure 7B through 7E demonstrates that the INE may correspond to the slow pathway. The absence of Cx43 from much of the INE (Figure 2D) can help explain the slowness of conduction. In the rabbit, we have recently reported that the INE also does not express the cardiac Na+ channel, Nav1.5 (unlike nearby atrial and ventricular muscle)11; this explains the slow upstroke of the action potential at the INE in the rabbit (<≈12 V/sec)10 and can also help explain the slowness of conduction. The INE also expresses a...
different L-type Ca\(^{2+}\) channel isoform, Ca,1.3, as compared with the atrial and ventricular muscle (which expresses Ca,1.2).\(^{11}\)

If the INE is indeed the slow pathway, it must have entry and exit sites for the action potential. Retrograde conduction during reentry was visualized in the preparation used to construct the model, and during this, the action potential propagated retrogradely along the slow pathway and exited into the atrial muscle (Figure 7D and 7E). The action potential appeared to exit from the slow pathway (presumably the INE) at the isthmus (as also observed by Nikolski and Efimov\(^{6}\)), and Figure 1C shows that at this site there is a possible connection between the INE and the atrial muscle. Of course, the INE is also continuous with the penetrating bundle (as shown in Figure 5C).

**Site of Pacemaking**

This study showed that during pacemaking at the AVN, the action potential is first initiated in the INE (Figure 7A). This is consistent with our earlier report.\(^{1}\) This is the logical site for pacemaking to occur; we have recently shown that mRNA for HCN4, the major isoform responsible for the pacemaker current \(I_{h}\), is more abundant in the INE than in other tissues (eg, penetrating bundle).\(^{11}\) HCN4 protein is also highly abundant in the INE.\(^{1}\) The INE also shares many other features in common with the SAN (eg, absence of Cx43 and Na,1.5, as well as the expression of Ca,1.3).\(^{11,12}\)

**Nature of Fast Pathway**

Electrophysiologically, the fast pathway is believed to be made of transitional atrio-nodal cells.\(^{9}\) The fast pathway is cranial: via this pathway, the action potential enters the INE immediately before the penetrating bundle (Figure 1B). Figures 3A and 5 show that, at this point, the nodal tissue makes contact with transitional tissue, and, therefore, the transitional tissue could constitute the fast pathway. Therefore, tissue that is transitional anatomically may be transitional electrophysiologically. Anatomically, the tissue is transitional, because it is similar to atrial muscle in terms of neurofilament and Cx43 expression but is similar to nodal tissue in terms of myocyte diameter and packing. Recently, we have obtained some evidence of reduced Na,1.5 and KChIP2 expression in this tissue, and, perhaps, this could help explain the unique electrophysiological characteristics of the atrio-nodal cells making up the fast pathway.\(^{11}\)

**Reentry**

The slow pathway is known to be involved in AVN reentry. If the INE is the slow pathway (and for it to be involved in reentry), apart from its entry and exit points, it should be electrically isolated from the surrounding tissue. Of course, it is possible that the electric isolation is functional. However, in the present study, evidence was obtained of a possible structural basis for the isolation of the INE: between the entry and exit points, there appears to be little connection between the nodal tissue and surrounding transitional tissue/atrial muscle; the nodal tissue is isolated by connective and adipose tissue and most importantly by a vein (Figures 1D and 5E).

Using the anatomic model, if we assumed that, between the entry and exit points, there is little connection between the nodal and transitional tissue/atrial muscle, we were able to simulate reentry (Figure 8B). Clinically, reentry is stopped by ablation of the isthmus\(^{2}\); Figure 8B shows why and supplemental Movie 4 confirms this.

At the end of the INE, Figure 5C shows parallel Cx43-negative and Cx43-positive tracts of nodal tissue (overlapping red and purple regions). Could these be responsible for intranodal reentry? Running alongside the INE, there is a prominent compact bundle of atrial muscle (evident in both Figures 1D, asterisk, and 3A and 3B, asterisk). This bundle has been highlighted in Figure 5D. The atrial myocytes in this bundle generally run longitudinally along the bundle. Could this prominent bundle (not previously identified) play an important role in reentry?

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**Disclosures**

None.

**References**


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Methods

**Animals.** ~2 kg New Zealand White rabbits (provided by Washington University, St. Louis, USA or University of Leeds, UK) were sacrificed in accordance with the guidelines of the American Heart Association or according to the United Kingdom Animals (Scientific Procedures) Act, 1986.

**Electrophysiology.** The AVN preparation used to construct the model was first electrophysiologically mapped using fluorescent imaging. In brief, the preparation was stained with di-4-ANEPPS (1 µM in perfusate) and voltage-sensitive fluorescent signals were recorded from a 8×8 mm area of the AVN at a rate of 1500 frames/s using a 16×16 photodiode array as previously described. The excitation-contraction uncoupler, 2,3-butanediol monoxime (15 mM), was added to the perfusate to suppress motion artefacts. Signals were low-pass filtered at 120 Hz, differentiated, normalised by the basic beat recordings, and plotted as two-dimensional intensity graphs, which were overlapped as frames with the image of the preparation to produce animations. Wavefronts of activation were visualised in these animations in order to identify the anatomical location of the pacemaker and conduction pathways. The preparation was digitally photographed and the field of view was identified with an accuracy of 0.2 mm.

**Paraffin-embedding and sectioning.** Following electrophysiology, the preparation was paraffin-embedded: in brief, the preparation was fixed in 10% neutral buffered formalin for 24 h, washed in 70 % ethanol for 2 h and embedded in paraffin overnight. The paraffin-embedded preparation was sectioned: ~10 µm sections were cut and mounted on Superfrost Plus glass slides (BDH, Leicester, UK).

**Histology.** Paraffin-embedded sections at each of 65 levels were de-waxed in Histoclear (Fisher Scientific, Loughborough, UK), hydrated through graded ethanols (100 to 70 %), post-fixed in Bouin's fluid for 15 min, and washed three times in 70 % ethanol (each wash 10 min). The sections were stained with Masson's trichrome as previously described. After staining, sections were dehydrated through graded ethanols (70 to 100 %), cleared in Histoclear and mounted in DPX mounting medium (BDH) for permanent mounting. With the Masson's trichrome technique, nuclei are stained dark blue/black, myocytes are stained purple and connective tissue is stained blue. Masson's trichrome stained sections were stored at room temperature for subsequent viewing with a Leica Materials Workstation (Leica Microsystems, Wetzlar, Germany) or a Zeiss Axio Imager.Z1 microscope fitted with an AxioCam HRc camera and Axiovision software (Carl Zeiss, Germany). High definition images of the sections were collected by making a mosaic of images collected with a 10× objective.

**Antibodies.** Antibodies used were: (a) mouse monoclonal antibody to middle (160/165 kD) neurofilament - either MAB5254 (Chemicon, Harrow, UK) or 2H3 (Developmental Studies Hybridoma Bank, University of Iowa, USA); (b) mouse monoclonal antibody to connexin43 (Cx43) (MAB3068; Chemicon).

**Immunoenzyme labelling.** Prior to the immunoenzyme technique, sections from each of the 65 levels were dewaxed in xylene and treated with 100 % ethanol for 10 min. The sections were then treated with H2O2 in methanol (2 ml of 30 % solution of H2O2 per 100 ml of methanol) for 30 min. Sections were treated with an antigen unmasking solution (H-3300; Vector Labs, Peterborough, UK) according to the manufacturer’s instructions in a microwave for 10 min at boiling point. Sections were treated with 0.2 % Triton-X 100 diluted in phosphate buffered saline (PBS) for 30 min, washed in PBS three times (each wash 10 min) and blocked in normal horse serum (diluted in PBS according to the instructions in the Vectastain ABC kit, PK-6102, Vector Labs) for 60 min. Sections were labelled with the anti-neurofilament and anti-Cx43 primary antibodies for 24 h at 4°C. Each primary antibody was diluted in 1 % bovine serum albumin in PBS and used at a dilution of 1:100. After incubation in the primary antibodies, the sections were washed three times in PBS over 30 min, and incubated with biotinylated anti-mouse secondary antibody for ~2 h and washed again three times in PBS over 30 min. Sections were incubated in ABC reagents for 60 min and this was again followed by three washes in PBS over 30 min. The biotinylated anti-mouse secondary antibody and ABC reagents were prepared according to the instructions in the Vectastain ABC kit. Sections were developed in DAB solution for 5-10 min. DAB solution was prepared according to the instructions in the Vector Peroxidase Substrate Kit (SK-4100; Vector Labs). Finally, sections were washed in distilled H2O for 10 min, dehydrated in graded ethanols (50 to 100 %), cleared in xylene, and mounted in a permanent mounting medium (VectorMount; H-5000; Vector Labs). No labelling above background was obtained when the primary antibodies were omitted (data not shown). Immunoenzyme labelled sections were stored at room
temperature for subsequent viewing with the Leica or Zeiss systems. High definition images of the sections were collected by making a mosaic of images collected with a 10× objective.

**Measurement of myocyte orientation.** The Masson’s trichrome stained sections were inspected under the microscope with a 40× or 63× objective. The approximate myocyte orientation was determined manually: myocytes with a round shape were assumed to be running perpendicular to the plane of the section (e.g. Fig. S1A); myocytes with a long elliptical shape were assumed to be running in the plane of the section (e.g. Fig. S1B).

**Three-dimensional (3D) reconstruction of the AVN.** Corel Draw (version 11.0; Corel Corporation, Ontario, Canada) and MATLAB (version 6.5; The MathWorks, Inc., Matick, MA, USA) were used to analyse the images and construct a 3D anatomical model of the AVN. There were six steps in this process.

**Step 1. Segmentation.** In the Masson’s trichrome stained sections, various tissue types could be identified: myocytes were stained purple, connective tissue was stained blue and fatty tissue was white. On the images of the Masson’s trichrome stained sections, Corel Draw was used to outline the various tissue types (carried out manually) and highlight them with a colour unique to that cell type. The result was two-dimensional “model sections” showing the distribution of myocytes and connective and fatty tissue.

**Step 2. Further segmentation.** The Masson’s trichrome stained sections were compared with adjacent neurofilament and Cx43 labelled sections to distinguish different myocyte types: (i) neurofilament-positive, loosely-packed, Cx43-negative nodal tissue, (ii) neurofilament-positive, densely-packed, Cx43-positive nodal tissue, (iii) neurofilament-positive, loosely-packed, Cx43-positive nodal tissue, (iv) neurofilament-negative, loosely-packed, Cx43-positive transitional tissue, (v) neurofilament-negative, densely-packed, Cx43-positive atrial muscle and (vi) neurofilament-negative, densely-packed, Cx43-positive ventricular muscle. This information was incorporated into the model sections.

**Step 3. Correction.** When the shape of a section was unchanged, but the section was affected by some combination of translation, rotation and scaling, linear conformal transformation was used. Let the x axis be from right to left, the y axis be from ventral to dorsal, the z axis be from caudal to cranial and

\[
sc = scale \cdot \cos(\text{angle})
\]

and

\[
ss = scale \cdot \sin(\text{angle}).
\]

Then

\[
[u \ v] = \begin{bmatrix} sc & -ss \\ ss & sc \\ tx & ty \end{bmatrix} \cdot \begin{bmatrix} x \\ y \end{bmatrix},
\]

where x and y are the coordinates of the input section we want to transform and u and v are the coordinates of the output section. Equation 1 was solved for sc, ss, tx and ty. At least two control-point pairs are needed to solve for the four unknown coefficients.

**Step 4. Alignment.** Alignment of the model sections was crucial to model development. The tract of nodal tissue, tendon of Todaro, coronary sinus, and the bottom edge of the preparation were the main landmarks used to align the sections as will be explained. Fig. S2 shows three screenshots from the custom written programme used for correction and alignment. The current model section being worked on is shown in colour in the bottom half of the screen; the previous section is shown in greyscale in the top half of the screen. In Fig. S2A, in the top half of the screen, the outline of the current section is shown in red. This demonstrates that the correspondence between the current section and the previous section is good. In Fig. S2B, in the top half of the screen, the outline of the tendon of Todaro in the current section is shown in red; again the correspondence between the tendon of Todaro in the current and previous sections is good. In Fig. S2C, in the top half of the screen, the outline of the compact node in the current section is shown in red; again the correspondence between the compact node in the current and previous sections is good. After all the sections had been aligned in this way, the envelope of the model sections was superimposed on a photograph of the original preparation (Fig. S3). Note that the starting and end points of the sectioning (in relation to the original preparation) were known. The bottom edge of the original envelope of model sections is shown by the dotted line \( f(z) \), nonlinear function) in Fig. S3 and it does not correspond exactly to the bottom edge of the original preparation (solid line: \( g(z) \), nonlinear function). The alignment of the sections was next corrected: let

\[
ty(z) = g(z) - f(z);
\]

then
Step 5. Regularising the array. The original sections were at variable interval. Piecewise linear interpolation was used to interpolate model sections to obtain model sections at a regular 50 \( \mu \)m interval; this increased the number of model sections from 65 to 174. Step 6. Visualising the model. The isosurface method was used to view the model. The 3D array model (File 1) is available as part of the Online Data Supplement.

**Cellular automaton model.** We simulated the spread of activation using the cellular automaton model. The cellular automaton model, although simple, is a useful model to calculate the activation sequence in the heart. The anatomical model consists of ~13 million ‘nodes’. Each node is equivalent to a cluster of myocytes or connective tissue. According to the cellular automaton model, each of the ~13 million nodes in the anatomical model at time, \( t \), is assigned a state, \( u_i(t) \), where the subscript refers to the node number (Fig. S4A). Each node can be in one of three states: resting, excited or refractory. If \( u_i(t) = 0 \), myocyte node \( i \) is in its resting state and can be excited by neighbouring myocyte nodes. However, as expected, myocyte node \( i \) cannot excite neighbouring myocyte nodes. The definition of neighbouring nodes to node \( i \) is shown in Fig. S4B. If node \( j \) is in an ellipsoid centred on node \( i \), node \( j \) is defined as a neighbouring node of node \( i \) (Fig. S4B). However, if there are connective tissue nodes between node \( i \) and \( j \), node \( j \) is not defined as a neighbouring node of node \( i \). The use of an ellipsoid (Fig. S4B) allows for the anisotropic properties of cardiac tissue. We assumed an anisotropic ratio of the radii in the longitudinal direction (parallel to the long axis of myocytes) and the two transverse directions (perpendicular to the long axis) – i.e. \( R_L : R_T \)- of 3:1 (Table S1). In the anatomical model, myocyte orientation was assumed to vary throughout the AVN conduction axis as shown in Fig. S4C (a simplified version of myocyte orientation based on Fig. 6).

The conduction velocity varies throughout the atrioventricular junction: the conduction velocity of the atrial muscle of the triangle of Koch in the rabbit is 35±17 cm/s, whereas the conduction velocity of the ‘N region’ (possibly the inferior nodal extension) in the rabbit is 2-10 cm/s. In large part, this is likely to be the result of changes in electrical coupling in different regions of the AV junction. Pollack reported that, in the rabbit, coupling (assessed by dye transfer between cells) between AVN myocytes was at least three orders of magnitude lower than between atrial myocytes. Electrical coupling is provided by connexins. Connexin43 (Cx43) forms 60–100 pS gap junction channels and is abundant in the atrial muscle of the triangle of Koch in the rabbit. In contrast, Cx45, which forms 20–40 pS gap junction channels, is expressed in the atrial muscle of the triangle of Koch in the rabbit. Connexin43 (Cx43) forms 60–100 pS gap junction channels and is abundant in the atrial muscle of the triangle of Koch in the rabbit. Connexin43 (Cx43) forms 60–100 pS gap junction channels and is abundant in the atrial muscle of the triangle of Koch in the rabbit. Connexin43 (Cx43) forms 60–100 pS gap junction channels and is abundant in the atrial muscle of the triangle of Koch in the rabbit. Connexin43 (Cx43) forms 60–100 pS gap junction channels and is abundant in the atrial muscle of the triangle of Koch in the rabbit. Connexin43 (Cx43) forms 60–100 pS gap junction channels and is abundant in the atrial muscle of the triangle of Koch in the rabbit. Connexin43 (Cx43) forms 60–100 pS gap junction channels and is abundant in the atrial muscle of the triangle of Koch in the rabbit. Connexin43 (Cx43) forms 60–100 pS gap junction channels and is abundant in the atrial muscle of the triangle of Koch in the rabbit.

If \( \frac{P_r}{E_i + P_r} < u_i(t) \leq 1 \), myocyte node \( i \) is in its excited state (where \( E_i \) and \( P_r \) are the excitable period and the refractory period, respectively). \( E_i \) was assumed to be 5 ms. If myocyte node \( i \) is in its excited state, node \( i \) is able to excite neighbouring myocyte nodes. If \( 0 < u_i(t) \leq \frac{P_r}{E_i + P_r} \), myocyte node \( i \) is in its refractory state and can neither be excited by neighbouring myocyte nodes nor excite neighbouring myocyte nodes. For resting myocyte node \( i \) to switch into the excited state, two requirements must be met: \( u_i(t) = 0 \), i.e. myocyte node \( i \) must be in the resting state; and \( \sum_j e_j > \theta_i \), i.e. the sum of the excitatory electrotonic current from neighbouring cells must be greater than a critical threshold, \( \theta_i \) (assumed to be 1). The refractory period, \( P_r \), is known to vary among the tissues of the AVN conduction axis: atrial muscle<slow pathway<fast pathway (Table S2). This was incorporated into the model (Table S3; Fig. S4D).

**Monodomain model.** We also simulated the spread of activation using the monodomain model. With the monodomain model, biophysically-detailed models of the action potential were used. For atrial muscle, the Lindblad et al. model of the rabbit atrial action potential was used. For the rabbit AVN, based on experimental data, we have recently developed a family of action potential models. At the rabbit AVN, three types of myocyte have been identified: true nodal N-type myocytes, transitional AN-type myocytes...
(with properties between those of the N myocytes and the atrial myocytes) and transitional NH myocytes (with properties between those of the N myocytes and the myocytes of the bundle of His). For example, atrial myocytes possess Na⁺ current ($I_{Na}$) and lack the pacemaker current ($I_{f}$) and consequently have a fast action potential and do not exhibit pacemaking. In contrast, AVN myocytes with N-type action potentials generally lack $I_{Na}$ and possess $I_{f}$ and consequently they have a slow action potential and many of them exhibit pacemaking. Distinct electrophysiological features of myocytes with AN- and NH-type action potentials have also been reported. We developed models for all three myocyte types. We assumed AN myocytes make up the transitional zone, N myocytes make up the inferior nodal extension and NH myocytes make up the penetrating bundle. This is consistent with ion channel expression in these three regions: the inferior nodal extension has the most nodal type ion channel expression profile (e.g. it lacks Naᵥ1.5, responsible for $I_{Na}$, and it expresses HCN4, responsible for $I_{f}$), whereas the transitional zone and penetrating bundle have a more transitional ion channel expression profile (e.g. it is likely that they express some Naᵥ1.5 and less HCN4).

Within the inferior nodal extension, $g_{Na}$ was assumed to change in a sigmoidal fashion:

$$g_{Na} = \frac{0.5 \times 10^{-12}}{1.0 + \exp\left(\frac{x - 5.6}{-5.0}\right)},$$  (2)

where $x$ is the distance (in mm) from the left side of the anatomical model.

The excitable behaviour of cardiac tissue was modelled as a continuous system using the following partial differential equation, usually referred to as the monodomain model:

$$\frac{\partial V}{\partial t} = \frac{1}{C_m} \left( I_{ion} + I_{stim} \right) + \left( D_x \frac{\partial^2 V}{\partial x^2} + D_y \frac{\partial^2 V}{\partial y^2} + D_z \frac{\partial^2 V}{\partial z^2} \right),$$  (3)

where $C_m$ is the membrane capacitance, $I_{ion}$ is the sum of ionic currents, $D_x$, $D_y$, and $D_z$ are diffusion coefficients in the x, y and z planes, and $I_{stim}$ is stimulus current. $I_{stim}$ was a current pulse 10 nA in amplitude and 1 ms in duration and it was injected into the atrial muscle of the crista terminalis. The diffusion coefficients are a measure of electrical coupling. We assumed an anisotropic ratio of the diffusion coefficients in the longitudinal direction (parallel to the long axis of myocytes) and the two transverse directions (perpendicular to the long axis) – i.e. $D_L:D_T$ of 3:1 (Table S1) – compare to the use of the cellular automaton model. Once again, in the anatomical model, myocyte orientation was assumed to vary throughout the atrioventricular conduction axis as shown in Fig. S4C (a simplified version of myocyte orientation based on Fig. 6). $D_L$ and $D_T$ were varied between tissues (Table S1) to vary the electrical coupling. It was assumed that the electrical coupling of atrial muscle > transitional zone > inferior nodal extension > penetrating bundle. $D_L$ and $D_T$ were varied manually (not iteratively; c.f. 14) to obtain conduction velocities consistent with those measured in experiments.

All simulations were coded in C++ and MPI (message passing interface) and were run on 40 nodes of a computer cluster with the Linux operating system. A Runge-Kutta numerical integration method (RKF45) was used to solve the ordinary differential equations. The time step was 5 μs, which gave a stable solution of the equations and maintained the accuracy of the computation of membrane current and potential. The voxel size of the anatomical model was reduced to 100×100×100 μm to make the model tractable (100 μm is close to the length of myocytes).

**Measurement of local conduction velocity.** An algorithm was used to determine the local conduction velocity vector. Let the spatial coordinates of a node be $x$, $y$ and $z$ and its activation time be $t_{x,y,z}$. At the node, the conduction velocities in the three planes ($v_x$, $v_y$, and $v_z$) were calculated by dividing the distance from the previous node to the next node by the activation time delay between the two nodes:

$$v_x = \frac{2\Delta x}{t_{x+1,y,z} - t_{x-1,y,z}}, v_y = \frac{2\Delta y}{t_{x+1,y,z} - t_{x-1,y,z}}, v_z = \frac{2\Delta z}{t_{x,y+1,z} - t_{x,y-1,z}}.$$  (4)

where $\Delta x$, $\Delta y$ and $\Delta z$ are the distances (100 μm) between two nodes in the three planes. The conduction velocity at $(x, y, z)$, $v_{x,y,z}$, is defined as follows,
\[ \mathbf{v}_{x,y,z} = v_x \mathbf{i} + v_y \mathbf{j} + v_z \mathbf{k}, \quad (5) \]

where \( \mathbf{i}, \mathbf{j} \) and \( \mathbf{k} \) are unitary vectors in the \( x, y \) and \( z \) directions, respectively. The magnitude of the conduction velocity, \( |v_{x,y,z}| \), is defined as follows,

\[ |v_{x,y,z}| = \sqrt{v_x^2 + v_y^2 + v_z^2}. \quad (6) \]

**Limitations of the study**

In the present study, myocyte orientation was only measured approximately and in the future it should be measured accurately with a technique such as diffusion tensor MRI. The structure-function relationships of the anatomical model need to continually refined as we discover more about the expression of gap junctions and ion channels in the AVN, nervous innervation of the AVN, and remodelling of the AVN in disease. Although the behaviour of the AVN in isolation is important, the behaviour of the AVN should also be understood in the context of the whole heart – currently, we are developing an anatomical model of the right atrium including the SAN and AVN.

**Sources of Funding**

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**References**


9. Greener ID, Tellez JO, Dobrzynski H, Yamamoto M, Billeter-Clark R, Boyett MR. Distribution of ion channel transcripts in the rabbit atrioventricular node as studied using in situ hybridisation and quantitative PCR. *J Mol Cell Cardiol.* 2006;40:982-983.


Movies and File

**Movie 1. Animation of the 3D anatomical model of the AVN.** See Fig. 5 for a key to the colours. During the course of the movie, different cell types are removed to reveal underlying structures.

**Movie 2. Simulation of anterograde conduction through the AVN using the monodomain model.** The preparation was stimulated at the interatrial septum as shown by the stimulating electrodes. There is a flash and click coincident with the stimulus.

**Movie 3. Simulation of fast-slow reentry using the cellular automaton model.** Responses to S1 and S2 stimuli are shown. S1-S2 interval, 96 ms. The preparation was stimulated at the His bundle as shown by the stimulating electrodes. There is a flash and click coincident with each stimulus. Same data as shown in Fig. 8B,C.

**Movie 4. Simulation of slow-fast reentry and the effect of slow pathway ablation.** Cellular automaton model used. Responses to S1 and S2 stimuli are shown. S1-S2 interval, 96 ms. The preparation was stimulated at the crista terminalis as shown by the stimulating electrodes. There is a flash and click coincident with each stimulus. Left, slow-fast reentry under control conditions. Same data as shown in Fig. S10. Right, abolition of reentry by slow pathway ablation. The ablation site is shown. In each panel, the timing of the stimuli is shown at the top left and the timing of action potentials at the recording electrodes on the His bundle is shown at the bottom right.

**Model Array Data.txt. The 3D anatomical model of the AVN.** The model is an array consisting of ~13 million nodes. The model is saved as a 250 MB text file (Model Array Data.txt). The text file has been converted to an 8 MB Microsoft cabinet file (Model Array Data.cab). To view the text file, we recommend using Microsoft WordPad. The text file lists the x, y and z coordinates and the cell type of each node. 1, fat; 2, connective tissue; 3, ventricular muscle; 4, atrial muscle; 5, aortic valve; 6, transitional tissue; 7, tendon of Todaro; 8, inferior nodal extension; 9, penetrating bundle; 10, vein; 11, compact node.
**Table S1.** Coupling parameters used with the anatomical model of the AVN. L and T refer to the longitudinal and two transverse axes of a myocyte. $g_j$, coupling conductance.

<table>
<thead>
<tr>
<th></th>
<th>Atrial muscle (peach)</th>
<th>Transitional tissue</th>
<th>Inferior nodal extension (red)</th>
<th>Penetrating bundle (purple)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellular automaton model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$R_L$</td>
<td>630 µm</td>
<td>630 µm</td>
<td>90 µm</td>
<td>360 µm</td>
</tr>
<tr>
<td>$R_T$</td>
<td>210 µm</td>
<td>210 µm</td>
<td>30 µm</td>
<td>120 µm</td>
</tr>
<tr>
<td><strong>Monodomain model</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$D_L$ (equivalent $g_j$)</td>
<td>1.25 cm²/s (625 nS)</td>
<td>0.4 cm²/s (160 nS)</td>
<td>0.1 cm²/s (29 nS)</td>
<td>0.4 cm²/s (160 nS)</td>
</tr>
<tr>
<td>$D_T$ (equivalent $g_j$)</td>
<td>0.417 cm²/s (208 nS)</td>
<td>0.133 cm²/s (53.3 nS)</td>
<td>0.033 cm²/s (9.67 nS)</td>
<td>0.133 cm²/s (53.3 nS)</td>
</tr>
</tbody>
</table>

**Table S2.** Refractory periods at the rabbit AVN.

<table>
<thead>
<tr>
<th>Source</th>
<th>Atrial muscle</th>
<th>Fast pathway</th>
<th>Slow pathway</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. Billette (unpublished data)</td>
<td>81±5 ms</td>
<td>127±9 ms</td>
<td>91±12 ms</td>
</tr>
<tr>
<td>Reid et al.17</td>
<td>-</td>
<td>141±15 ms</td>
<td>91±10 ms</td>
</tr>
<tr>
<td>Lin et al.18</td>
<td>-</td>
<td>-</td>
<td>100±9 ms</td>
</tr>
</tbody>
</table>

**Table S3.** Refractory periods ($E_i+P_i$) used in the cellular automaton model.

<table>
<thead>
<tr>
<th>Atrial muscle (peach)</th>
<th>Transitional tissue (green)</th>
<th>Inferior nodal extension (red)</th>
<th>Penetrating bundle (purple)</th>
</tr>
</thead>
<tbody>
<tr>
<td>81 ms</td>
<td>134 ms</td>
<td>94 ms</td>
<td>94 ms</td>
</tr>
</tbody>
</table>
Figure S1. Measurement of myocyte orientation. Two high magnification images of the Masson’s trichrome stained section corresponding to the boxed regions in Fig. 6A are shown. A shows transitional tissue next to the tendon of Todaro (blue; tT). The majority of the myocytes (purple) appear to be sectioned transversely (resulting in a circular cross-section) and the myocytes are assumed to be running perpendicular to the section. B shows (from left to right) transitional tissue, nodal tissue and the central fibrous body (blue; CFB). The majority of myocytes appear to be sectioned longitudinally (resulting in a long elliptical cross-section) and the myocytes are assumed to be running in the plane of the section (at an angle of ~45°).
Figure S2. Three screenshots from the custom written programme used for correction and alignment. The current model section being worked on is shown in colour in the bottom half of the screen; the previous section is shown in greyscale in the top half of the screen. A, outline of the current section (shown in red in the top half of the screen). B, outline of the tendon of Todaro in the current section (shown in red in the top half of the screen). C, outline of the compact node in the current section (shown in red in the top half of the screen).
Figure S3. Envelope of the model sections superimposed on a photograph of the original preparation. Dotted line, bottom edge of the original envelope of model sections. Solid line, bottom edge of the original preparation.
Figure S4. Cellular automaton model. A, time course of state \((u)\) of node \(i\). If the node is excited, the node state is changed from 0 (resting) to 1 (excited). The node state then declines back to 0. B, definition of neighbouring nodes of node \(i\). If node \(j\) is an ellipsoid centred on node \(i\), node \(j\) is defined as a neighbouring node of node \(i\). The parameters, \(R_x\), \(R_y\), and \(R_z\), determine the shape of the ellipsoid and, therefore, account for the anisotropy of cardiac tissue (the dependence of conduction velocity on cell orientation) and coupling conductance. C, D, myocyte orientation (C) and refractory periods (D) in the anatomical model of the AVN used in the simulation of action potential conduction. In C, the arrows show myocyte orientation.
Figure S5. Nerve trunk at the inferior nodal extension. A, Masson’s trichrome stained section. B, adjacent neurofilament labelled section. From box B in A.
Figure S6. Masson-trichrome stained sections at the 65 levels on which the 3D anatomical model is based – part 1. Distances in mm shown (see Fig. 1B). Note the densely-packed bundle of atrial muscle on the right hand side of the preparation immediately above the central fibrous body extending from 2.36 to 4.66 mm (at least).
Figure S7. Masson-trichrome stained sections at the 65 levels on which the 3D anatomical model is based – part 2. Distances in mm shown (see Fig. 1B).
Figure S8. 65 model sections used to construct the 3D anatomical model of the AVN. The whole of the sections are shown. Distances in mm shown (see Fig. 1B).
Figure S9. Local conduction velocities. Left, magnitude of local conduction velocity. Right, direction of local conduction (shown as arrows). The data are calculated from the simulation shown in Fig. 8A.
Figure S10. Simulation of slow-fast reentry using the cellular automaton model. A, B, simulation of the activation sequence during slow-fast reentry at the AVN. The preparation was stimulated using a S1-S2 protocol (S1-S2 interval, 96 ms). The isochrones (at 5 ms intervals) and yellow arrows show the propagation of the action potential: A shows the propagation of the S1 action potential and B shows the propagation of the S2 action potential. C, extracellular potentials recorded at the crista terminalis (at high and low sites) and His bundle of the rabbit AVN conduction axis during slow-fast reentry (top; S1-S2 interval, 110 ms; from Nikolski et al.1) and equivalent signals from equivalent sites in the simulation of slow-fast reentry (bottom; from the simulation shown in A and B). In both experiment and simulation, the crista terminalis (high) was the stimulation site.