# Morphology of the atrioventricular junction in *Iguana iguana* (Reptilia-Iguanidae)

Sonia Regina JURADO[1]
Roberto Jorge da Silva FRANCO[2]
Valdívia Rocha MORCELI[3]

1 - Department of Natural Sciences, Federal University of Mato Grosso do Sul, Três Lagoas - MS
2. Department of Medical Clinic, State University of São Paulo, Medical Faculty of Botucatu, Botucatu - SP
3. Department of Biology, Laboratory of Ecology, Federal University of Mato Grosso do Sul, Três Lagoas - MS

**Correspondence to:**
SONIA REGINA JURADO
Departamento de Ciências Naturais
Universidade Federal de Mato Grosso do Sul
Campus Universitário de Três Lagoas
Av. Ranulpho Marquês Leal, 3484 - Distrito Industrial
Caixa Postal 210.
79620-080 - Três Lagoas - MS
srjurado@bol.com.br

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## Abstract

The atrioventricular junctional area (AVJA), including atrioventricular (AV) node and bundle was investigated in seven hearts of common or green iguana (*Iguana iguana*) using the light microscopy. Adult animals, both sexes, were captured in the Pantanal, Brazil. All hearts were fixed in buffered formaldehyde 10% (pH 7.2) for 24 hours, embedded in paraplast according to routine methods, and serially cut at 5 ?m thickness. In the *Iguana iguana*, the AVJA consists of a mass of the fibers intermingled with variable amount of connective tissue and blood vessels surrounded by adjacent myocardium and the attachment of the right atrioventricular valve in the fibrous skeleton. By light microscopy, conducting cells of the AV node and bundle can be distinguished from working cells by their much smaller size, paler staining reaction and the presence of a sheath of connective tissue. The AV node and bundle and its branches were found to constitute a continuous tract. Histochemically, we found elastic fibers between cells of the conduction, mainly in the AV node. The PAS method reveals absence of glycogen in specialized cells. The fibrous skeleton, mainly the right trigone, showed a well-developed chondroid tissue, made by hyaline like cartilage (binucleated condrocytes included in the big lacunas and extracellular matrix with fibrillar collagen). In conclusion, the nodal and Purkinje cells in heart iguana presented poorly morphological differentiation comparing mammals and birds, however the skeleton fibrous has a different cartilage kind.

**Key-words:**
Atrioventricular junction.
Node atrioventricular.
Purkinje cell.
Heart.
Iguana iguana.

## Introduction

Numerous references have been made to the marked species variability to be found in the conduction tissue disposition. Entire monographs have been devoted to this difference, such as that of Chiodi and Bertolami[1], while Lev[2] and Treux and Smythe[3] have prepared excellent reviews of the differing morphology. While the anatomy of human conduction tissue is the most studied, information concerning animal conduction tissues is also significant and most of knowledge of the eletrophysiology, enervation, histochemistry and ultraestruture of conduction tissue comes from of these species. In this context, it must be emphasized that each species has it own disposition of conduction tissues, so that results obtained in one animal species are not necessarily applicable or reproducible in another species[4].

The light microscopical studies can demonstrate wide variation the structures of the hearts, from one species to another, and even within the same species[5]. At this magnification, Truex and Symthe[3] divide the higher animals into three categories in terms of the clarity of differentiation of the conducting system. It is generally well differentiated in birds and some mammals (echidna, platypus, whale, cow, deer, sheep, pig) and poorly in reptiles and some rodents (bat, rat, guinea, pig, rabbit). In between these extremes, there is a group where the differentiation is moderate; this group includes squirrel, cat, monkey, gorilla and man.

The specialized tissues of the heart that initiate and conduct the cardiac impulse consist of three major parts: (1) sinus node (pacemaker, node of Keith-Flack); (2) atrioventricular junctional area (AVJA), including node of Tawara (node of Aschoff-Tawara) and His bundle (penetrating bundle, AV Bundle, common bundle) and (3) bundle branches (branching portion of AV bundle, bifurcation, ventricular conduction tissue) and terminal Purkinje fibers[6].

Controversy still exists about whether impulses from sinus node to the AV node travel over specialized conducting "pathways" or over nonspecialized plain atrial myocardium. In the poikilotherms this impulse is transmitted by the normal cardiac muscle fibers but in the homeothermic animals this specialized conducting system facilitates the spread of the stimulus from the sinoatrial node[7].

The conduction system consists of a series of muscle cells specialized for the conduction of impulses rather than contraction. These fibers are usually rich in glycogen and striated only periphery[8,9]. The sinus node, first reported by Keith and Flack[10] is easily recognized a low magnification, histologically. Specific cell types in the sinus node include nodal cells, transitional cells and atrial muscle cells. Nodal cells are small, pale standing, and poorly striated to general myocardial cells[11]. Transitional cells (T cells) are elongated cells with characteristic intermediate between the packed nodal cells and the individual atrial cells[12].

The atrioventricular node (AV node), discovered by Tawara[13], it is composed of a thick mesh of tiny pale cells, which anastomose with one another by short pluridirectional projections of their cytoplasm ("star cells")[14]. These cells are interwoven with collagen and elastic fibers. At the electron microscopic level, James and Sherf[15] examined human hearts and described four types of AV nodal cells: pale cells (P cells), transitional cells, common myocardial cells, and Purkinje cells. The latter are large and vacuolated located in the subendocardial region but have not the same location found in all species[16].

The morphology of conduction tissues have been studied in rat[17,18], rabbit[19], dog[20], guinea pig[21], ungulate[22] however, data referring to morphological descriptions in lizard heart are limited.

An arboreal lizard, the common or green iguana, *Iguana iguana* is a exciting specie to study conduction system because the heart rate is extremely oscilable, the zero when it is diving up 110 beats per minute during activity[23,24]. This specie has a total length of 90-180 centimeters, with the tail accounting for two-thirds of the length. This animal is bright green with dark stripes on the tail and it has a serrated dewlap under the throat and crest of soft spines running from one end of the body to the other. It is a tree living, strictly vegetarian found in the forests of tropical America[25].

The aim of the present work is to study the atrioventricular junctional area (AVJA) in *Iguana iguana* at the level of light microscopy.

## Materials and methods

Seven adults from both sexes of green iguanas, *Iguana iguana* (Figura 1), of mean mass 1500 g and measuring 111-150 cm in rostrum-anal length, were caught with IBAMA (Brazilian Institute of the Environment and Natural Resources) permission under renewed license n? 012/2001 and process n? 020.140.077/01-10.

These animals were captured from October 2000 through September 2001 in their natural state, on the Miranda River banks or along forest in this river, at Pantanal Study

Center of Federal University of Mato Grosso do Sul (latitude 19?34'36" S; 57?01'06" W), State of Mato Grosso do Sul, Brazil.

In the period of study, summer was characterized by rains and winter by their absence. Peak rainfall occurred during December 2000 and January 2001 (around 1.000 $mm^3$). Air temperature ranged from 21 to 32? C; the hottest months were December, January and February, and the coldest June and July. The vegetation is tropical forest, with trees bordering the river.

To the sacrifice, the lizards were anaesthetized with sulfuric ether inhalation and thoracotomized to remove the hearts (Figura 2). The hearts were longitudinally sectioned (Figura 3) and fixed by immersion in buffered formaldehyde 10% (pH 7.2) for 24 hours. The samples were dehydrated in ethanol, embedded in paraplast and cut at 5?m thick sections. Deparafinized sections were stained by Hematoxylin-Eosin, Mallory's thrichrome stains, van Gieson, Mallory Azan, Picro Sirius and periodic acid-Schiff (PAS).

## Results

The heart of *I. iguana* is approximately oval, more long than broad. The interventricular septum is largely horizontal and divides the ventricle into dorsal and ventral cavities, rather than right and left sides. The dorsal ventricular chamber is more extensive than the ventral chamber. At the apex of the ventricle, the septum is complete but toward the ventricular base the septum assumes a more oblique position and has a defect that allows communication between the dorsal and ventral chamber.

The atrioventricular node lies in the base of the interatrial septum deep to the endocardium and fibrous skeleton. It has an elongated shape with fibrous connective tissue stroma, several large venules and small arterioles (Figura 4). The vessels, adjoining bundles of nerves and ganglion cells, and the relationship of the node to fibrous trigone are landmarks that aid in the identification of the AV node.

The common AV bundle, in its lowest part, bifurcates in the left and right bundles. The cells of the bundles branches are large, vacuolated and with few myofibrils (Figura 5). The left AV branch is longer than right branch (Figura 6). The latter is related with right atrioventricular valve (Figura 7).

The component fibers of AV node consist of nodal fibers, which have a pale, partially empty appearance because of the paucity of myofibrils. Nodal cells have a central nucleus with a circular or oval profile. The proximal AV bundle has an admixture of nodal and large, pale fibers. Farther distally most of the muscle fibers in the AV bundle assume a large diameter or have a more abundant cytoplasm and some fibers may demonstrate a peripheral palisading of the myofibrils. Cords of fibrous tissue, the chordae tendineae were localized in the heart. The chordae may run directly from ventricular wall into the fibrous skeleton (Figura 8).

A fibrous tissue well developed in the heart of the animal, it affords a firm anchorage for the attachments of atrial and ventricular musculature as well as the valvular tissue. The atrioventricular junction area is enclosed by fibrous skeleton, which has a fibrocartilaginous character.

The chondroid tissue was composed by hyaline like cartilage with some binucleated condrocytes included in big lacunas (Figura 9). The PAS method showed positive reaction in the cartilage, due to the presence of neutral mucins (glycoproteins) (Figure 9). The cartilaginous matrix also stained with the Sirius red, suggesting the presence of fibrillar collagen.

## Discussion

There are two major anatomical arrangements of the heart in modern reptiles. This first is found in the lizards and the


Figure 1. The common or green iguana (Iguana iguana), an arboreal lizard found in the Pantanal, Brazil

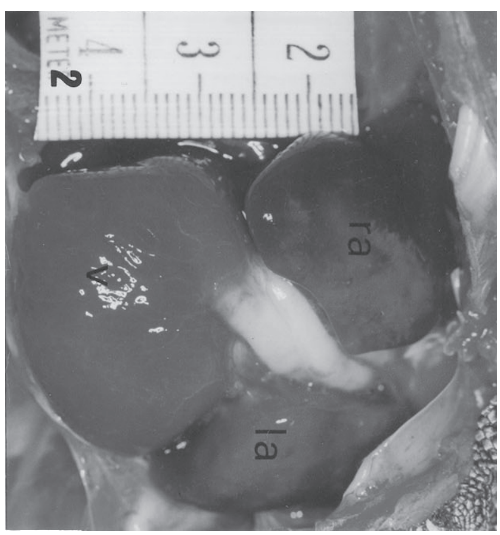


Figure 2. Gross anatomy of Iguana heart in situ. RA and LA = right and left auricles, respectively. V = ventricle

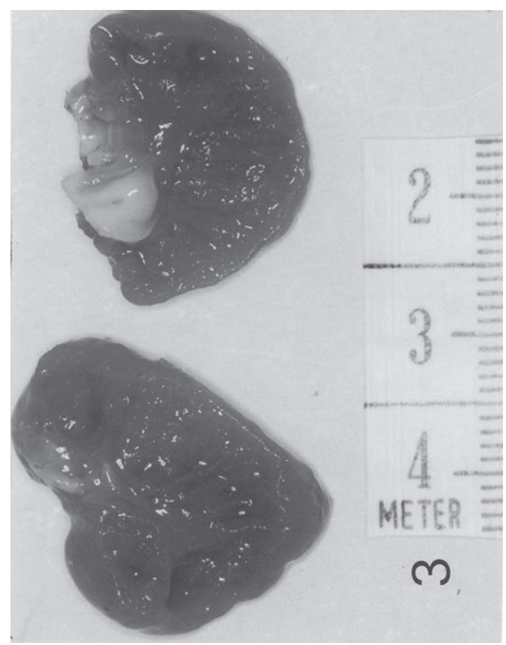


Figure 3. Iguana heart longitudinally sectioned

second in crocodiles[7].

Lizard ventricles are not completely separated by septum, unlike crocodiles and mammalian ventricles. Separation of oxygenated and deoxygenated blood within the ventricle depends largely on the flow patterns within the ventricle[24]. The possibility of the right-to-left and left-to-right shunts exists in lizards ventricles[26], but the extent to which this actually occurs is not clear.

In crocodiles, the heart has a complete interventricular septum dividing the ventricle into right and left sides. The anatomy of the crocodilian heart suggests that it is similar to that birds and mammals[7]. Iguanas did not demonstrate any shunting in one study[27],

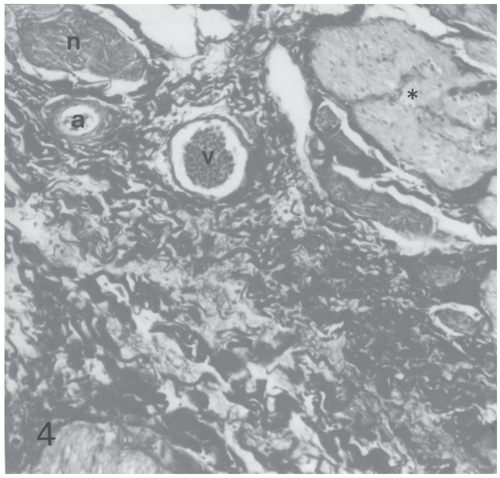


Figure 4. Histological section at level atrioventricular node. Note the pale cells (asterisk), small arteriole (a), large venule (v) and bundles of nerves (n) nervosas. Van Gieson stain, x 100

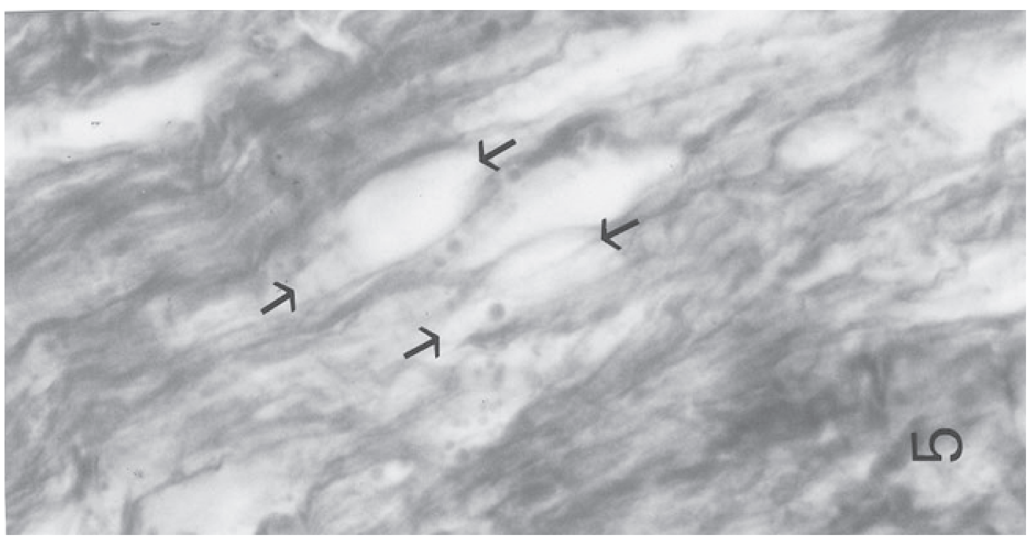


Figure 5. Subendocardial pale cells, vacuolated and with few myofibrils (fibers like Purkinje) (arrows). Mallory's trichrome stain, x 400

while 50 and 75% of the iguanas in two other studies[28,29] show shunting right-to-left.

The direction of these shunts is related to the balance between the resistance to flow in the systemic and pulmonary circulations. When the animal is actively respiring, pulmonary resistance is relatively low; there is a distinct left-to-right shift so that some blood from the pulmonary return may be redirected to the lungs. When the animal is diving and pulmonary resistance is high, there is a right-to-left shunt so that blood may be rerouted to the systemic circulation, instead of the entering the pulmonary circuit[7].

In comparison with mammals and birds, the conducting system is poorly differentiated in the heart reptile[5]. However, it is possible to give meaningful descriptions of the various elements in the heart of *I. iguana*.

The atrioventricular node is localized in the base of the interatrial septum, closely applied to the skeleton fibrous in mammals[3], unlike iguanas.

In this study, we found all components of the conducting system, AV node and its bundle and the beginning of the right and left branches well as a part of fibrous skeleton in heart of the iguana. These components also noted in mammal heart[3,8,30,31,32,33,34].

In the AV node of the iguana heart, the node cells are organized less compact way than in mammals. In these, the node presents compact (central) and transitional (peripherally diffused closely related to the interatrial septum) regions[35]. However, unlike mammals, the nodal fibers in the AV node of iguana are arranged in longitudinal and transverse planes.

The light microscopic cellular characteristics in the AVJA described to iguana, is virtually the same in other species,

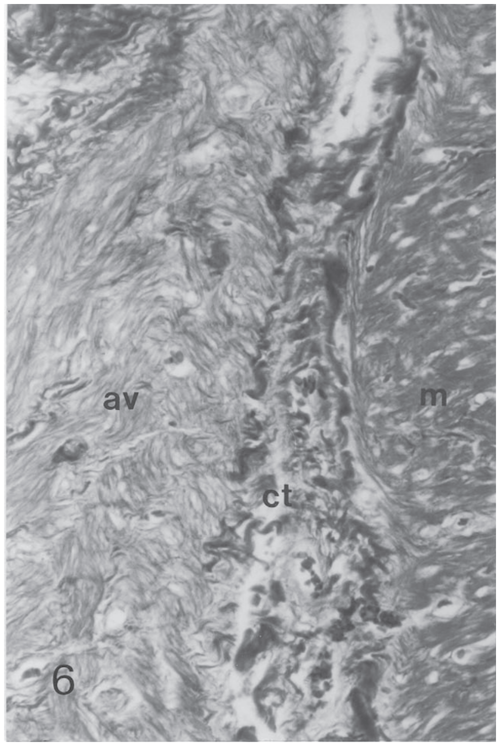


Figure 6. Photomicroph of the right atrioventricular bundle (av), it has conducting cells interwoven with connective tissue (ct). These fibers have paler staining comparing with the working cells (m). Mallory's trichrome stain, x 100

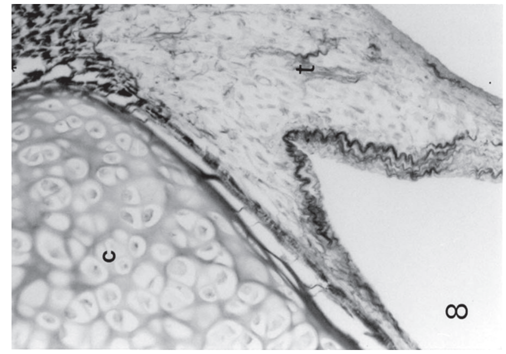


Figure 8. Fibrous trigone compose by hyaline like cartilage (c) and fibrous tissue (asterisks). A chordae tendineae is inserted in the fibrous trigone (t). Mallory's trichrome stain, x 400

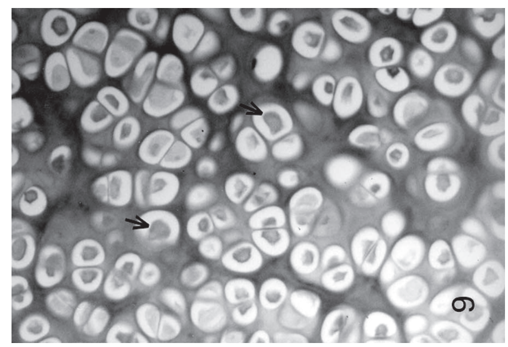


Figure 9. Hyaline like cartilage has condrocytes included in big lacunas (arrows). The cartilaginous matrix stained with the Sirius red, suggesting the presence of fibrillar collagen (in red) (x 1000)

such as bats[3,35], guinea pig[21], rabbit[36], monkey[8,33,34], opossum, armadillo, squirrel, cat, rat[37,38], sperm whale[39] and man[36].

The cells of the bundle branches have been described as Purkinje or Purkinje-like cells[31,40]. Purkinje described the classical Purkinje fibers in ungulate's heart in 1845. These fibers comprise the final link between the bundle branches and the ventricular cardiac muscle fibers, and the like common bundle, manifest wide variations from specimen to specimen and species to species[3].

In the present study, these cells have clear differentiation in the conducting system. These are bigger and paler than the working cells because its low myofibrilar content[5].

The extreme variations in Purkinje fibers are responsible for much of the confusion to be found in the literature. In general, these fiber components of the conducting system fall into three broad categories in the animals studied[5].

The first category of the fibers has large diameters, is well differentiated. Ventricular conduction fibers of the second category may approximate the diameter of the large fibers but most of them are of intermediate or small size, have more myofibrils, and often resemble the cardiac fibers of the ventricular myocardium. For this reason, such fibers are often described as being transitional. The third category consists of the presumptive conduction fibers that demonstrate little or no differentiation. Such endocardially-placed fibers are difficult to distinguish from the ventricular muscle fibers[3]. Purkinje like fibers in iguana may be included in the second category due to its intermediate differentiation, meanly in comparison with working cells in the myocardium. An abundant amount of connective tissue surrounded the AV node in iguana. In chiropteras, there also is considerable amount of connective tissue in this region as other mammals[35,41].

Curran[42] reported the sheath, which received his name, like a thin connective tissue sheath surrounding the AV node and bundle in man. In the studied specie in this work, the fibrous skeleton has a hyaline like cartilage, which differs a little from that cartilage in mammals. The cartilage of the fibrous skeleton in iguana has big lacunas and condrocytes, which are common in elastic cartilage, so, it resembles an intermediate cartilage type.

## Conclusions

The nodal and Purkinje cells in heart iguana presented poorly morphological differentiation comparing mammals and birds, however the skeleton fibrous has a different cartilage kind.

To a reptile, iguana has a well-developed junction atrioventricular what can be relationed with its cardiac physiology.

## Morfologia da junção atrioventricular em *Iguana iguana* (Reptilia-Iguanidae)


### Resumo

A área da junção atrioventricular (AJAV), incluindo nó e feixe atrioventriculares foi investigada em sete corações de iguana comum ou verde (*Iguana iguana*), usando microscopia de luz. Animais adultos, de ambos os sexos, foram capturados no Pantanal, Brasil. Todos os corações foram fixados em formaldeído tamponado 10% (pH 7,2) por 24 horas, incluídos em parapast de acordo com métodos de rotina e seccionados com 5?m de espessura. Na iguana, a AJAV consiste de uma massa de fibras musculares mergulhada em uma quantidade variável de tecido conjuntivo e vasos sangüíneos, rodeada por miocárdio adjacente e aderida à valva atrioventricular direita no esqueleto fibroso. Através da microscopia de luz, células de condução do nó e feixe atrioventriculares podem ser distinguidas das células de trabalho por serem menores, de coloração pálida e pela presença de um envoltório de tecido conjuntivo. O nó AV bem como o feixe AV e seus ramos formam um trato contínuo. Histologicamente, encontramos fibras elásticas entre as células de condução, principalmente no nó AV. O método do PAS revelou a presença de glicogênio nas células especializadas. O esqueleto fibroso, principalmente, o trígono direito, apresentou um tecido condróide bem desenvolvido, constituído de cartilagem semelhante à hialina (condrócitos binucleados inseridos em grandes lacunas e matriz extracelular com colágeno fibrilar). O esqueleto fibroso tem fibras colágenas e cartilagem semelhante à hialina. Em conclusão, as células nodais e de Purkinje no coração de iguana apresentam pouca diferença morfológica quando comparadas às de mamíferos e aves, contudo o esqueleto fibroso tem um tipo diferente de cartilagem.



**Palavras-chave:**
Feixe atrioventricular.
Nó atrioventricular.
Célula de Purkinje.
Coração.
Iguana iguana.


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