Conduction barriers and pathways of the sinoatrial pacemaker complex: their role in normal rhythm and atrial arrhythmias

Vadim V. Fedorov, Alexey V. Glukhov, and Roger Chang

Department of Physiology and Cell Biology, and Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University Medical Center, Columbus, Ohio

Submitted 8 September 2011; accepted in final form 18 January 2012

Fedorov VV, Glukhov AV, Chang R. Conduction barriers and pathways of the sinoatrial node; their role in normal rhythm and atrial arrhythmias. Am J Physiol Heart Circ Physiol 302: H1773–H1783, 2012. First published January 20, 2012; doi:10.1152/ajpheart.00892.2011.—Since Keith and Flack’s anatomical discovery of the sinoatrial node (SAN), the primary pacemaker of the heart, the question of how such a small SAN structure can pace the entire heart has remained for a large part unanswered. Recent advances in optical mapping technology have made it possible to unambiguously resolve the origin of excitation and conduction within the animal and human SAN. The combination of high-resolution optical mapping and histological structural analysis reveals that the canine and human SANs are functionally insulated from the surrounding atrial myocardium, except for several critical conduction pathways. Indeed, the SAN as a leading pacemaker requires anatomical (fibrosis, fat, and blood vessels) and/or functional barriers (paucity of connexins) to protect it from the hyperpolarizing influence of the surrounding atrium. The presence of conduction barriers and pathways may help explain how a small cluster of pacemaker cells in the SAN pacemaker complex manages to depolarize different, widely distributed areas of the right atria as evidenced functionally by exit points and breakthroughs. The autonomic nervous system and humoral factors can further regulate conduction through these pathways, affecting pacemaker automaticity and ultimately heart rate. Moreover, the conduction barriers and multiple pathways can form substrates for reentrant activity and thus lead to atrial flutter and fibrillation. This review aims to provide new insight into the function of the SAN pacemaker complex and the interaction between the atrial pacemakers and the surrounding atrial myocardium not only in animal models but also human hearts.

sinoatrial node; optical mapping; atrial fibrillation; atrial flutter

Introduction

In 1910, Wybauw (52) and Lewis et al. (37), using string galvanometers, observed that the site of origin of the mammalian heartbeat coincided with the location of the sinus node located at the junction of the superior vena cava (SVC) and right atria (RA), anatomically described by Keith and Flack in 1906 (31). These studies were the first functional evidence that the sinus node or sinoatrial node (SAN) was the primary pacemaker of the heart. Several years later, Meek and Eyster (39) studied the dynamic changes of the heart rate and the origin of atrial excitation in a dog heart through vagal stimulation or by atrial cooling. They observed that heart rate slowing usually correlated with a shift in the origin of excitation from the SAN to an area inferior to the node along the crista terminalis (CT) or even to the atrioventricular (AV) node. These studies were the first demonstration that the right atria could have multiple origins of excitation during “sinus” rhythm.

Since then, numerous epicardial (7, 16) and endocardial (13) mapping studies demonstrated anatomically widespread sites of earliest atrial depolarization, which could occasionally activate simultaneously. These points of origin were reported to arise at the epicardial and/or endocardial region along the CT, over a region that spans 7.5 cm in length (7, 13). This area was significantly greater than the SAN structure itself (27, 50). To help explain this relationship between the SAN structure and function, two possible mechanisms were put forward by Boineau et al (7) and Schuessler (45). The first mechanism proposed that the multifocal activity of atria was caused by a widely distributed system of pacemakers. The alternative mechanism proposed the existence of “a unique form of specialized conduction within the intramural layers of the crista terminalis,” which can poten-
tially deliver depolarization waves simultaneously to the different areas of atrial myocardium (Fig. 1).

For more than 20 years these two putative mechanisms remained untested because of the inability of epicardial and endocardial electrode mapping to detect the origin as well as the slow propagation of excitation within the intramural structure of the SAN (10, 23, 45). Thus the atrial pacemaker complex remained an enigma, which led to the current perception of the SAN as a simplistic, single dominant pacemaker located in the right atrium near the SVC. However, recent integrative studies that combined optical mapping techniques and detailed histology revealed what truly occurs in this “black box” of the human heart. The SAN is actually a very complex heterogeneous structure, consisting of multiple pacemaker compartments and conduction pathways, with varying electrophysiological properties. In particular, recent studies of the canine and human SAN (21) and AV (19) nodes delineated the necessary structural substrates that provided insight into the mechanisms of initiation of electrical activation in the pacemaker complex and subsequent propagation to the atria (Fig. 1). This review highlights current as well as past research on the role of the specialized structure of the SAN pacemaker complex in mammalian hearts.

**New Model of the Canine and Human SAN Pacemaker Complex**

The mammalian SAN is a specialized and complex structure (9, 27, 42). Anatomical (10, 27, 29, 49, 50) and functional (7, 46) studies suggest that the canine SAN (23) is a more realistic model for the human SAN (21) than that of small mammals (9, 22). Figure 1 represents a more recent view of the structure of SAN pacemaker complex in canine and human hearts that consists of several major pacemaker compartments (head, center, and tail) surrounded by coronary arteries, fibrous tissue, fat, and the atrial myocardium all situated along CT. The presented model was developed from recent (20, 21, 23) as well as past canine and human SAN studies (11, 15, 21, 27, 31, 50) that used a comprehensive combination of functional, structural, and molecular methodologies. Recently, it has been demonstrated that the canine and human SAN complex has at least four preferential conduction pathways (SACPs), which are responsible for the transmission of electrical impulses to atrial myocardium.

During normal sinus rhythm, excitation originates in one of these pacemaker compartments and slowly propagates through SAN tissue. It continues through different SACPs and excites...
the atria at different exit points, which marks the beginning of the P wave on the electrocardiogram (SAN electrical activation could not be detected by regular ECG in dog and human hearts). Depending on the preferential SACP (inferior or superior), the excitation wave spreads from the right atria to the left atria through the main intraatrial musculature: either through the Bachmann’s bundle (2, 13) and the coronary sinus musculature (1, 12) or through anterosuperior and posteroinferior intra-atrial septum connections (44).

Figure 1A shows that the SAN pacemaker complex is electrically coupled to the atrium only through these SACPSs. It is electrically insulated from other areas by structural and functional conduction barriers. These conduction barriers are important components of the SAN pacemaker complex, preventing depression of the pacemaker automaticity from the hyperpolarizing electrical load of the atrial myocardium (33). This idea of an uncoupling SAN pacemaker complex is supported by a computer simulation of SAN as a two-dimensional sheet of cells (30). This simulation revealed that electrical uncoupling of cells within the SAN from those without may be an essential feature of normal electrical communication between the SAN and the atrium.

Since the following sections of this review address the detailed components of this model (Fig. 1), particularly the conduction barriers and the SACPs, the reader is encouraged to refer back to the model outlined in Fig. 1.

Pacemaker Shift Versus Preferential Conduction Pathway

The concept of widely distributed “atrial pacemaker complex” was first introduced in the canine heart (8) and was later illustrated in the human heart by Boineau et al. (7) using atrial epicardial multielectrodes mapping. However, despite multi-electrode mapping, the authors were unable to detect the actual origin of excitation or determine the conduction pathways within the intramural structure of the SAN before it activated the adjacent atrial myocardium. This limitation of surface electrode mapping was overcome by optical mapping with voltage-sensitive dyes, which was able to resolve the origin of activation and conduction inside of intramural SAN structure. Such high resolution is possible because optical action potentials (OAPs) represent a weighted average of transmembrane signals containing fluorescent signals from myocardium up to a depth of several millimeters (4, 17, 19). In Fig. 1, B–D, the SAN model explains multiple observations made by electrode and optical mapping. For instance, canine optical measurements of the membrane potentials (20) directly revealed that isoproterenol (Iso) accelerated sinus rhythm and produced a superior shift of the leading pacemaker inside of the SAN complex, whereas acetylcholine (ACh) had the opposite effects (Fig. 1B). Moreover, perfusion with Iso and ACh generally results in a preferential use of superior and inferior SACPs, respectively, because of intranodal pacemaker shift and/or inhomogeneous changes in conduction within the SAN.

The inferior and superior SACPs could have different conduction properties and different sensitivity to autonomic stimulations by ACh and Iso (5, 20). Moreover, ACh, adenosine, ischemia, and/or structural remodeling lead not only to depression of SAN pacemaker function but also to the complete block of conduction through all SACPs (Fig. 1D). This exit block within SAN allows pacemaking to originate from other regions such as the pulmonary vein, “ring of the fire,” (53) or other latent pacemakers [e.g., AV junction (19) and Purkinje fibers]. Thus SACPs play an important role in regulating SAN pacemaker automaticity and ultimately in maintenance of heart rate.

Taken together these findings directly support the Boineau-Schuessler hypothetical model of the SAN with its conduction pathways (45). They are in agreement with the concept of a pacemaker hierarchy within the SAN with the fastest pacemakers located at the superior part (head) and the slowest at the inferior part (tail). The presented model is also in agreement with earlier studies of SAN pacemaker synchronization conducted by the Jalife group (14, 40). These studies demonstrated that electrical coupling plays a crucial role for synchronization of pacemaker clusters inside of the SAN. Consequently, inhomogeneous changes in the coupling between pacemaker clusters and their excitability could lead to pacemaker shift and multiple pacemaker activity.

Functional Evidence of SAN Conduction Pathways in Canine Hearts

Even though optical mapping may offer new insights into SAN pacemaker and conduction properties relative to multi-electrode mapping, optical recordings could be affected by scattering in epicardial fat and connective tissue in the SAN region. The complexity of the optical recordings coupled to this limitation serve as a point of contention vis-à-vis resolution of SAN intramural conduction (17). To address this issue, Fig. 2 provides a direct comparison between the “gold standard” of cardiac electrophysiology, microelectrodes recordings (10), and OAPs from the canine SAN.

More specifically, Bromberg et al. (10) demonstrated with surface electrode mapping and microelectrode recordings from the SAN that the earliest activation in the atrial myocardium or “atrial breakthroughs” did not correlate with the sites of earliest activation within the SAN (Fig. 2A). In this study, the authors concluded that the sites of earliest atrial activation represented sites of excitation exit from a relatively insulated SAN. This observation was directly supported by recent optical mapping techniques (20, 21, 23, 26). Figure 2B shows an example of canine SAN and atrial OAPs obtained during recovery from pacing-induced SAN exit block (26, 26). During the exit block, the morphologies of SAN OAPs in Fig. 2B exhibit the same patterns as microelectrode recordings in Fig. 2A recorded by Bromberg et al. (10). In both examples, the entire SAN activates after more than 200 ms because of very slow conduction. In Fig. 2B, two components of the OAP upstrokes were detected and separately used to reconstruct the activation of the SAN and atria. The SAN activated the atria through two superior SACPs at two exit points (atrial breakthroughs), superior and lateral to the earliest SAN activation. Previously, Bromberg et al. (10) made a very important observation that during normal sinus rhythm, the peripheral parts of the SAN (see superior SAN recordings from Fig. 2A) could be activated significantly later than the atrial myocardium. It meant that the atrium was activated before SAN activation was completed. Optical mapping further supported the findings that peripheral SAN area was activated after the activation of the atria and was masked by atrial excitation. The SAN component of OAP could also be detected after the atrial component, which is demonstrated in the superior SAN OAP recording in Fig. 2B.
The results presented in Fig. 2 highlight that OAP recordings go hand in hand with the microelectrode recordings from the SAN.

While this elaborate specialized SAN conduction system is present in the clinically relevant canine model, it is important to address whether SACPs and conduction barriers are also present in humans.

**Functional Evidence of SAN Conduction Pathways in Human Hearts**

Figure 3A shows a summary of epicardial electrode mapping in the human atrium depicting widely distributed areas of earliest atrial depolarization sites (exit points) (7). The area of earliest atrial depolarization is significantly larger than the earliest atrial depolarization sites (exit points) (7). The area of the human SAN that was optically mapped (see Fig. 3A) (right). The SAN activation maps demonstrated that these two different atrial activation patterns corresponded to atrial activations by the SAN excitation waves exiting through superior and inferior SACPs rather than two different atrial pacemakers. Conduction in the SACPs could slow down because of a source-sink mismatch (43). A weak source of current in the relatively narrow exit pathway must excite the large load of the hyperpolarized funnel-like expansion of the atrial muscle.

However, any functional observation of the SACPs and conduction insulations around SAN require structural conformation, which will be presented below.

**Structural Evidence for Conduction Pathways and Barriers Between Atrial Myocardium and the SAN**

The first structural study of the human SAN by Keith and Flack (31) demonstrated the presence of connective tissue layers between the SAN and atrial myocardium (Fig. 4A). Figure 4B shows a histological section (21) of the superior part of the human SAN that was optically mapped (see Fig. 3B). The superior part (SAN head) lies about 1 mm beneath the epicardial layer of fat and connective tissue (No. 5 in Fig. 4A). The fibrotic layer (No. 6) labels a fibrotic barrier between the SAN and atrial myocardium. These conduction barriers were observed by many groups in both the human and canine SAN, and it was composed of connective...
tissues, fat, coronary arteries, and abrupt changes in the main gap junction protein, connexin-43 (Cx43), expression between atrial and nodal cells [Fig. 4, C (56) and D (23)]. Figures 4B also shows a SACP (No. 7), which was consistently observed in all human and dog SANs (21). These SACPs are represented by elongated and branching fibers of transitional cells, which formed a specialized area for preferential conduction for electrical impulse from the SAN to atrial myocardium. These findings as well as histological and immunohistochemical sections from other SAN preparations suggest that the SACPs are specialized, branching, muscular strips which contain transitional cells with Cx43 and cells without Cx43. While still functionally uncharacterized, these transitional cells’ electrophysiological properties may be a mixture of SAN and atrial cells.

The anatomical presence of a conduction barrier between SAN and atrial myocardium toward the septum and the CT is supported by classical histology studies published by the Truex, James, and Demolin groups.

First, in their 1965 review on p. 21, Truex and Smythe (49) wrote that the identification of the SAN is most easily done by looking at the surrounding mesh of connective tissues. They commented about the block zones found at the border of the node. “The ramifications and boundaries of the SA node are readily identified in most sections. They appear in sharp contrast to the larger and more deeply stained fascicles of atrial cardiac muscle.”

Second, in 1966 James et al. (29) investigated the ultrastructure of the SAN in humans and dogs, and they found that “Transitional cells, which have features intermediate between...”
P cells and working myocardium, serve as the connections between P cells and the rest of the heart. The aforementioned transitional cells make up the elongated and branching SACPs observed in our recent studies (21, 23).

Third, in 1978, Demoulin and Kulbertus (15) conducted a postmortem histological study of patients with SAN dysfunctions and observed that “areas of nodal atrial continuity are consistently seen along the margins of the compact sinus node (Truex, 1976). Such areas of blending of nodal and atrial muscle cells are notably found along the tail of the node, along its anterior aspect, and along its endocardial margins, the regions presumed to be the site of origin of the bundles described by Thorel (1909), Bachmann (1916), and Wenckebach (1906–1907) respectively. These areas most probably represent important structural bridges between the node and the atrial myocardium and were, therefore, carefully studied.”

Consequently, all these structural studies as well as molecular/structural studies (10, 34) support a new view that canine and human SAN tissues have clear structural/functional barriers between atrial myocardium except at the SACPs (Fig. 1A). However, the molecular markers for the

---

**Fig. 4.** Histological evidence of the conduction pathways and barriers between the SAN and atrium. A: Keith and Flack’s 1907 drawings of the SAN anatomy (red outline). A, left: drawing of the human atrial anatomy from the posterior side of the heart. A, right: drawing of a histological cross section from the line in left shows the following anatomical points of interest in the SAN region: 1, musculature of superior vena cava or sinus; 2, artery and surrounding musculature between the SAN (red) and atrium; 3, venous valve; 4, atrial muscle; 5, subepicardial fat tissue; and 6, connective fibrotic tissue. From Keith and Flack. (31); used with permission. B: histological slide from Fedorov et al. (21) of the human SAN preparation shown in Fig. 3B. The outlines of the SAN (red) and the SACP (yellow, 7) are shown, surrounded by fibrosis (purple, 6). C: immunohistochemistry of hyperpolarization-activated cyclic nucleotide-gated channel 2 (HCN2) and connexin-43 (Cx43) proteins expression near the canine SAN-atria border. From Zicha et al. (56); used with permission. D: transmural canine SAN histology sections (a, b, and c) made perpendicular to CT and about 1 mm apart from each other. Fibrosis covers the SAN entirely in a and c, but the SACP connects the SAN to the atrium in b. From Fedorov et al. (23); used with permission.
transitional cells that form the SACP are not clear and require further studies.

SAN and Atrial Tachyarrhythmias

The structure and function of the SAN pacemaker complex also appear to play an important role in the initiation and maintenance of atrial flutter and fibrillation (AFL and AF, respectively) (6, 41, 48). Furthermore, the fast AF/AFL rate could lead to SAN dysfunction (18, 54). While significant progress has been made in elucidating basic mechanisms of AF/AFL over the years, the SAN’s role in inducing and maintaining these clinically prevalent arrhythmias have yet to be investigated.

One of the major observations made about the SAN activity in rabbits during AF was accomplished by Kirchhof and Allessie in 1992 (32). The authors discovered with the help of microelectrodes a high degree of SAN entrance block (5:1) during low potassium-induced AF (32). However, this study (32) did not directly assess SAN activation during atrial arrhythmias because of the technical boundaries of simultaneously recording electrical activity from multiple microelectrodes. Recent studies of the clinically relevant canine model of autonomic-induced AF/AFL have overcome these limitations (20). It was demonstrated that during atrial tachypacing or AF/AFL, the SACPs acted like a low-pass filter for atrial waves by slowing conduction and creating entrance block. Autonomic stimulation (ACh or Iso) modulated these filtering properties of the SACPs by increasing or decreasing the degree of the entrance block, respectively. Moreover, cholinergic stimulation (ACh) depressed conduction through the SACPs, allowing the SAN to retain independent excitation patterns relative to the fast atrial pacing or reentrant activity. However, during β-adrenergic stimulation (Iso), the atrial waves capture the SAN and overdrive suppressed it.

This study (20) also revealed that the entire SAN structure along with its barriers creates a substrate for AFL by functioning as an obstacle around which atrial reentrant activity can be anchored in place. The authors proposed that the conduction barriers surrounding SAN, rather than anisotropy of atrial muscle, could underlie some forms of AFL observed in both dog and human atria. An outline of atrial flutter activation sequence observed with optical recordings in the canine atrium shows a classical type of AFL, which was shown for the first time in a dog heart by Lewis et al (36). Figure 5A shows the
interaction between the SAN and AFL reentry, which was observed in SACP. The SACP play an important role in protecting the SAN against overdrive activation during atrial arrhythmias. Moreover, the SAN can also play an active role in initiation or termination AF/AFL (47). For instance, cholinergic stimulation by ACh can induce unidirectional conduction block in the SACP and thus prevent AF/AFL impulses from suppressing the intrinsic SAN activity (Fig. 5B). Under these conditions, some SAN impulses might perpetuate the reentrant arrhythmias where AFL can be converted to AF or the SAN impulse can restore normal rhythm by interrupting the reentry. These studies illustrate the complex relationship between the SAN, SACP, autonomic nervous system, and surrounding atria. They also provide new insight into the mechanisms involved in AF/AFL as well as Tachy-Brady syndrome (26).

Mechanistic Insights from Genetically Engineered Mouse SAN Models

The mouse SAN is widely used as a genetic platform, allowing for modifications of the expression and/or function of different genes that encode the corresponding ion channels or Ca^{2+}-handling proteins. From numerous electrophysiological and histological studies, it can be concluded that the structure and function of the mouse SAN are simple representations of larger mammals. Microelectrode mapping by Verheijck et al. (51) demonstrated the electrophysiological features of the mouse SAN. The authors measured the size of the SAN as defined by the pacemaker action potentials to be within 300 μm parallel to the CT and 150 μm perpendicular to it. This area seems to represent the compact part of the SAN determined morphologically (38).
The pattern of the murine SAN activation obtained using optical mapping technique (Fig. 6A) was consistent with that revealed by microelectrode mapping (25), but this approach offered more insight into SAN function because of the improved spatial resolution relative to microelectrode recordings. Optical recordings from the mouse SAN exhibit the slow diastolic depolarization, slowly rising upstroke of the SAN (SAN component), and rapidly rising upstroke of the atrial myocardium (atrial component). Transitions of nodal and atrial waveforms occur over small distances (~100 μm) in the superior and inferior nodal area (enlarged in Fig. 6A, inset). From these experiments, the SAN conduction time from the area of earliest activation to the atrium was as long as 5 ms, which corresponds with microelectrodes measurements. It is important to emphasize that SAN excitation propagates with high anisotropy to the atrium myocardium with two preferential conduction directions near the superior and inferior SAN edges and complete block to the septal direction.

To reconstruct the structure of the murine SAN, Liu et al. (38) performed a detailed histology and immunolabeling staining of Cx43 and hyperpolarization-activated cyclic nucleotide-gated channel 4. Figure 6B shows sections through the mouse SAN perpendicular to the CT. A histology section shows that the compact part of the SAN is separated from the atrial muscle on either side by connective tissue, which may represent the conduction barriers similar to that observed in larger mammals (dogs and humans, Fig. 4). However, at a more distal section (0.7 mm), a specialized interface between the SAN and surrounding atrial muscle was identified: strands of hyperpolarization-activated cyclic nucleotide-gated channel 4-positive nodal cells protrude into the atrial muscle and strands of Cx43-positive atrial cells protrude into the SAN. Such small structures between the SAN and atrial muscle (Fig. 6A) could represent the preferential conduction pathways observed in the canine and human hearts (see Fig. 4) and may function similarly. Thus the combination of high-resolution optical mapping and histological structural analysis confirmed that the functional insulation of the mouse SAN was also required for normal pacemaking activity as shown for the canine and human SAN.

The normal mouse heart also demonstrates the presence of multiple pacemaker compartments distributed between the SVC and inferior vena cava (IVC) and between the CT and intraatrial septum. The importance of the functional anatomy of the entire SAN pacemaker complex is demonstrated in genetically engineered mouse models. Deletion of some proteins in the regulation of the cardiac pacemaker function [such as Cx40 (3, 35), ankryrin-B (24), and calsequestrin 2 (25)] results in a depression of the SAN function and a shift of the leading pacemaker outside of the SAN structure (Fig. 6A, green). Autonomic stimulation (25) or consecutive thermal ablation of such ectopic sites (3) resulted in leading pacemaker shift back to the SAN, but at a prolonged intrinsic cycle length. Moreover, a beat-to-beat competition between different pacemakers resulted in heart rate irregularities observed in these mouse models. The disorganized and widely distributed regions of the leading pacemaker sites observed in different genetically engineered mouse SAN models illustrate a dissociation within the pacemaker complex and can explain SAN dysfunction in individuals with similar familial mutations. These examples highlight the importance and potential of mouse heart optical mapping for SAN dysfunction studies.

Conclusion and Future Directions

Structural features of the SAN pacemaker complex are extremely important for its successful pacemaker function. SAN pacemakers clusters require anatomical (fibrosis, fat, and blood vessels) and/or functional (low connexin expression) barriers to protect them from the hyperpolarizing influence of the surrounding atrial myocardium. To overcome this source-sink mismatch pacemaker clusters require specialized conduction pathways. These pathways are extremely important in the modulation of heart rhythm and atrial arrhythmias. A recent study from Zhang et al. (55) supports the significance of such a specialized microanatomy for normal function of the SAN-based pacemakers. The authors demonstrated that 400,000 canine SAN cells implanted into the ventricle did not generate sufficient biological pacing function because of the different properties between the surrounding substrate and anatomic SAN. Therefore, both cellular and anatomical features of a natural pacemaker should be considered for the creation of a “biological pacemaker.”

Lack of a detailed understanding of SAN structural/functional complexity remains a significant critical barrier to our ability to optimally treat SAN dysfunction. The SAN pacemaker complex is a very complicated structure, and it is important to first consider the conduction from the leading pacemaker to the atrial myocardium through the SACPs. Continued studies of the SAN pacemaker complex using an integrative approach (high-resolution optical mapping combined with structural and molecular analysis) in the clinically relevant animal models will lead to the development of a novel genetic/cellular or device therapies for heart rhythm disorders.

ACKNOWLEDGMENTS

We thank Dr. Richard Schuessler, Dr. Przemyslaw Radwanski, and Dena Delaviz for valuable suggestions and critical comments.

GRANTS

This work was supported by Dorothy M. Davis Heart and Lung Research Institute start-up funding (to V. V. Federov).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author (s).

AUTHOR CONTRIBUTIONS

V.V.F., A.V.G., and R.C. conception and design of research; V.V.F. and A.V.G. performed experiments; V.V.F. and A.V.G. analyzed data; V.V.F., A.V.G., and R.C. interpreted results of experiments; V.V.F., A.V.G., and R.C. prepared figures; V.V.F., A.V.G., and R.C. drafted manuscript; V.V.F., A.V.G., and R.C. edited and revised manuscript; V.V.F., A.V.G., and R.C. approved final version of manuscript.

REFERENCES


