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The Left Atrial Ganglionated Plexus : Its Function and Pathways Relative to Atrial Fibrillation Surgery

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Resumé:

Le système nerveux autonome cardiaque est devenu une cible dans les thérapies ablatives de la fibrillation auriculaire. Nous avons étudié les voies de communication et la fonction des plexus ganglionnaires (PG) de l'oreillette gauche (PGOG) afin de clarifier la validité physiopathologique des méthodes de détection et des thérapies impliquant ces groupes de neuronnes. **Méthodes:** Vingt-deux chiens ont subi une double thoracotomie et ont été instrumentés avec des plaques auriculaires épidcardiques de multiélectrodes. Une stimulation électrique (2 mA, 15 Hz) des PGOG a été réalisée à l'état basal et successivement après: 1) une décentralisation vagale, 2) l'ablation par radiofréquence des plexus péri-aortiques et de la veine cave supérieure (Ao/VCS) et 3) l'ablation du PG de l'oreillette droite (PGOD). Ces procédures de dénervation ont été réalisées suivant une séquence antérograde (n = 17) ou rétrograde (n = 5). **Résultats:** Chez 17 des 22 animaux, la stimulation des PGOG a induit une bradycardie sinusale (149 ± 34 bpm *vs* 136 ± 28 bpm, $p < 0.002$) et des changements de repolarization (ΔREPOL) auriculaires isointégrales. Dans le groupe des ablations antérogrades, les réponses aux stimulations vagales ont été supprimées suite à la décentralisation vagale chez un seul animal, par l'ablation des plexus Ao/VCS dans 4 cas et par l'ablation du PGOG dans 5 autres animaux. Des changements ont persisté tout au long chez 2 chiens. La valeur de surface des ΔREPOL a diminué avec les dénervations séquentielles, passant de 365 ± 252 mm^2 en basale à 53 ± 106 mm^2 après l'ablation du PGOD ($p < 0.03$). Dans le groupe de dénervation rétrograde, les changements de repolarisation et chronotropiques ont été supprimés suite à l'ablation du PGOD chez deux chiens et suite à l'ablation Ao/VCS chez trois. La valeur de surface du ΔREPOL a aussi diminué après l'ablation du PGOD (269±144mm^2 *vs* 124±158mm^2, $p<0.05$). **Conclusion:** Les PGOD sont identifiables en préablation par la réponse bradycardique à la stimulation directe dans la plupart des cas. Le PGOD semble former la principale, mais non la seule, voie de communication avec le nœud sinusal. Ces résultats pourraient avoir des implications dans le traitement de la FA par méthodes ablatives.

Mots clés: Fibrillation auriculaire, système nerveux intrinsèque cardiaque, plexus ganglionnaire de l'oreillette gauche, neurocardiologie, système nerveux autonome.

Abstract

The cardiac autonomic nervous system has recently become the target of ablative therapy in the treatment of atrial fibrillation. We investigated the pathways and function of the left atrial ganglionated plexus (LAGP) to clarify the pathophysiologic validity of therapies involving this cluster of neurons. **Methods:** Twenty-two bilaterally thoracotomized canines were instrumented with atrial epicardial plaques. LAGP stimulation was performed in the basal state and successively following 1) vagal decentralization, 2) radiofrequency ablation of the peri-aortic/superior vena caval (Ao/SVC) plexi, and 3) of the right atrial ganglionated plexus (RAGP). Denervation was carried out in either the aforementioned order (n=17, antegrade) or reversed (n=5, retrograde). **Results:** In 17 of 22 animals, LAGP stimulation induced a sinus bradycardia (149±34bpm to 136±28bpm, $p<0.002$) and atrial isointegral repolarization changes (REPOLΔ). In the antegrade group, response was suppressed by vagal decentralization (n=1), Ao/SVC plexi ablation (n=4), and RAGP ablation (n=5). Changes persisted throughout in 2 canines. Surface area of REPOLΔ diminished with successive denervation, from 365±252 at baseline to 53±106mm^2 following RAGP ablation ($p<0.03$). With retrograde denervation, chronotropic and repolarisation changes were suppressed following RAGP ablation in two canines, and following Ao/SVC ablation in three. Surface area of REPOLΔ diminished following RAGP ablation as well (269±144mm^2 vs 124±158mm^2, $p<0.05$). **Conclusion:** The LAGP can be identified intraoperatively by the bradycardic response to direct stimulation in most cases. The RAGP appears to be the primary, but not the only, gateway to the sinus node. These results could have important clinical implications relating to ablative treatment of atrial fibrillation.

Key words: Atrial fibrillation, Intrinsic cardiac nervous system, left atrial ganglionated plexus, ablation, neurocardiology, autonomic nervous system, Atrial repolarisation, Isointegral mapping

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Abbreviations:

AF	Atrial Fibrillation
ANS	Autonomic nervous system
Ao	Aorta
AVN	Atrioventricular node
AVN	atrio-ventricular
BPM	Beats per minute
CL	Cycle length
CNS	Central nervous system
ICNS	Intrinsic cardiac nervous system
LAGP	Left atrial ganglionated plexus
RAFW	Right atrial free wall
RAGP	Right atrial ganglionated plexus
RAAS	Renin-angiotensin-aldosterone system
REPOLΔ	Repolarisation changes
SAN	Sinoatrial node
SVC	Superior Vena Cava

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Chapter 1. Atrial fibrillation and the Autonomic Nervous System

1.1 What is atrial fibrillation?

Atrial fibrillation (AF) is characterized by disorganized atrial electrical activity leading to rapid, irregular activation of the atrium. This activation can be in the order of 400-600 pulses per minute in a given segment of atrial muscle and results in asynchronous and inefficient atrial contraction.[1] It can be recognized on the surface electrocardiogram by the absence of the P wave (atrial wave), which is replaced by low amplitude irregular oscillatory waves, termed fibrillatory waves.[2] AF is the most prevalent cardiac arrhythmia in humans, being responsible for 33% of hospitalizations for cardiac rhythm disturbances. The prevalence has best been studied in the American population and is estimated to be 0.4-1% of the general population, with a larger proportion of the elderly being affected.[3] This corresponds to 2.2 million Americans and is likely to increase to 5.6 million by the year 2050 as a result of the aging population and increasing prevalence of cardiac disease.[4]

1.1.1 Classification

There have been several classification systems proposed for AF. One of the most commonly used in clinical practice is based on AF duration. If AF terminates spontaneously it is termed paroxysmal, while AF lasting 7 days or more is termed persistent. If it lasts more than 1 year it is designated permanent AF.[5] Dr Jim Cox, a pioneer in the surgical treatment of AF has proposed a simplified classification, with Atrial fibrillation being either paroxysmal or chronic.[6] He advocates this nomenclature because it will best separate patients according to the ideal therapeutic intervention. Another classification method is based on the underlying disease, for example, AF can be related to mitral stenosis, mitral regurgitation, or cardiomyopathy.[7] AF that occurs in the absence of identifiable concomitant cardiac disease is termed “lone AF”, accounting for 10-30% of AF cases.[7, 8] The autonomic nervous system has been implicated in the induction of AF due to imbalance, with

either parasympathetic or sympathetic hyperactivity.[9] It is generally recognized that AF occurring in the postoperative period following cardiac surgery falls into a separate category. Postoperative AF occurs following 11% to 40% of coronary artery bypass graft (CABG) surgeries and as many as 50% of cardiac valvular surgeries.[10] The onset of this subtype of AF is typically on the second or third postoperative day.[11, 12] It is thought to be related to inflammation resulting from surgical manipulation and extracorporeal circulation fluid shifts and endogenous and exogenous catecholamine release.[13-15] Although it is often a self limiting problem with 90% of patients returning to sinus rhythm at 6-8 weeks, it is associated a with significantly longer hospital stay as well as higher in-hospital and long term mortality.[16-18]

1.2 Clinical Implications

1.2.1 Morbidity and mortality

The clinical consequences of AF can be varied, ranging from slight discomfort due to palpitations to more severe complications such as stroke or death.[19, 20] This morbidity is related to the three different problems created by AF. Firstly, the chaotic atrial activation leads to an irregular ventricular response resulting in the sensation of palpitations, which can lead to patient discomfort.[21] These symptoms occur more frequently in patients with paroxysmal AF, that is, those having a rhythm that fluctuates between sinus rhythm and AF.[22] Secondly, stasis of blood in the left atrium can lead to the formation of clot within the heart and predispose a patient to thromboembolic events, resulting in damage to the kidneys, intestine, extremities or brain.[23, 24] For example, AF has been associated with a three to five time increase in the risk of stroke and as many as 30% of acute strokes occur in patients with AF.[20, 25] This can have severe consequences leading to significant impairment of quality of life or even death.[26] Finally, the loss of synchronous atrioventricular contraction can have important physiologic effects. Ventricular filling can be diminished thereby reducing exercise tolerance or leading to decompensated cardiac insufficiency by aggravating pre-existing compensated heart

failure.[27] AF with an ill-controlled ventricular response can also result in "tachycardia-induced cardiomyopathy" brought on by prolonged rapid ventricular rates, a condition that is for the most part reversible.[28] Overall, AF is associated with a 1.5 and 1.9 fold increase risk of death in men and women, respectively.[29]

1.2.2 Cost

Although it is difficult to determine the true financial impact of AF on the health care system, it is clear that it is a costly public health issue. A study from the United Kingdom suggested the direct cost of AF on the health care system in the year 2000 to be over 700 million dollars, accounting for almost 1% of total National Health Service expenditures. This was a 50% increase from the cost five years earlier.[30] Another European study estimated that AF costs the healthcare system between 2000$ and 3000$ per patient per year. With the high prevalence and increasing incidence of this disease, this amounts to an enormous burden on the healthcare system.[31]

1.3 **Risk Factors**

Although there are many factors associated with the development of AF, advanced age is the most significant independent risk factor. The exact cause for the increased prevalence in the elderly is incompletely understood but it is likely a combination of several phenomena associated with aging tissues, including atrophy of atrial muscle, atrial dilatation, and slowed or abnormal conduction.[32] Aside from age, other important risk factors include male sex, hypertension, congestive heart failure, atrial dilatation, and valvular disease.[33] Additional risk factors include coronary artery disease, recent myocardial infarction, hypothyroidism, sleep apnea, diabetes, obesity, metabolic syndrome and the postoperative state.[33-37] Many other factors have been identified that predispose a patient to atrial fibrillation following open-heart surgery, these include a history of atrial fibrillation, valvular surgery, and withdrawal from beta-blockers or ACE inhibitors[18, 38]

1.4 **Pathophysiology**

The pathophysiology and mechanisms of AF are complex and still incompletely understood. Despite this, beginning with a basic explanation of the fundamentals of the arrhythmia will permit a build-up to a comprehensive understanding of our knowledge to date. The onset of AF depends on the presence of two key elements: a *trigger*, i.e. an initiating event, and an anatomical substrate enabling AF *maintenance*[1]. The initiating event may result from a rapidly firing ectopic focus, multiple functional re-entrant circuits, or both.[1] Atrial histological and electrical changes allowing for AF maintenance may be pre-existing or may be induced by the presence of continually firing triggers causing atrial remodelling.

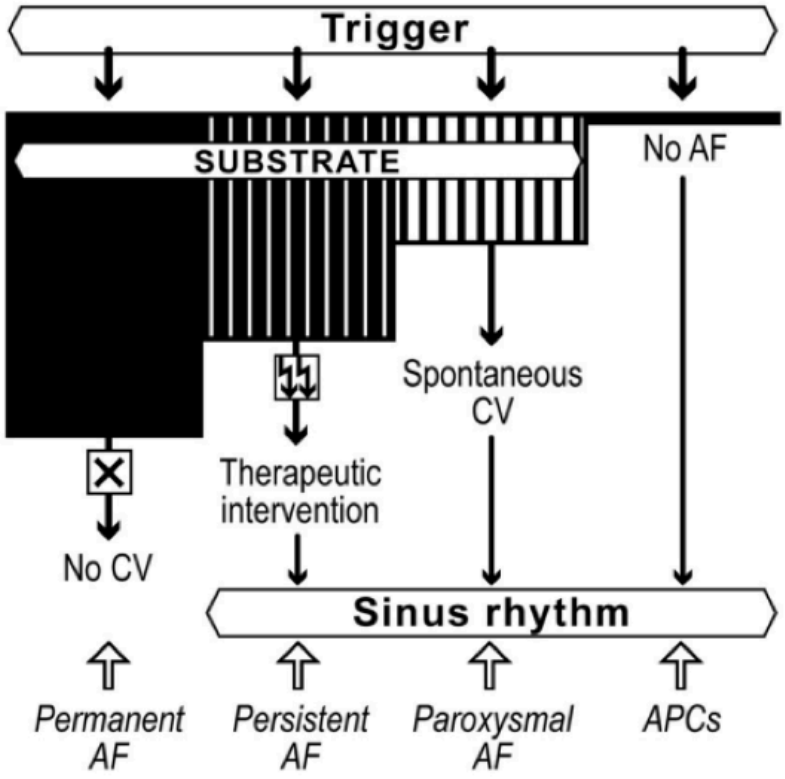

Figure 1. Relationship between the various forms of AF.

The trigger will initiate the re-entry phenomenon. If the anatomical or electrophysiological substrate is insufficient to perpetuate re-entry, the sequence will terminate with an atrial extrasystole (APC, atrial premature complex). In paroxysmal AF, the arrhythmia will be initiated by a trigger,

however, the substrate is insufficient to maintain AF and a spontaneous cardioversion ensues. In persistent AF the atrial substrate is sufficient to maintain a fibrillatory state but the arrhythmia can still be terminated with a therapeutic intervention. Permanent AF is present when severity of atrial remodelling makes it no longer possible to return to sinus rhythm. CV= Cardioversion[39]

1.4.1 Mechanisms of atrial fibrillation

In the early twentieth century three competing theories regarding the mechanisms of AF were suggested. They postulated that AF is derived from 1) rapidly discharging atrial ectopic foci, 2) a single re-entry circuit, or 3) multiple re-entry circuits.[40] These theories have been both disputed and modified over the years and have helped shape our current understanding of this complex arrhythmia. Whatever the exact mechanism, it appears that AF begins with abnormal impulses arising from atrial tissue. While normal automaticity results from the spontaneously depolarizing sinus node cell reaching threshold potential, ectopy can result either from other cells depolarizing at an abnormally rapid rate, or from afterdepolarizations reaching threshold and causing premature action potentials. Conversely, re-entry can occur as a result of a premature impulse propagating between two zones of tissue with different refractory periods.[1] In order for AF to be initiated, a premature activation must occur on the border between these two zones creating a unidirectional block when the impulse contacts one of the zones during its refractory period. Once the refractory period has terminated, the atrial tissue can then be re-excited and allow for propagation of the pathologic impulse. Once initiated, this circuit can continue indefinitely. With increased atrial tissue heterogeneity, the likelihood of unidirectional blocks occurring augments and the risk of chaotic activation leading to AF is amplified.[41-43]

These phenomena are the result of alterations in atrial ionic currents. Changes in the balance between the various potassium, sodium and calcium currents lead to varied action potential duration and a proarrhythmic state. As an example, in response to calcium overload, atrial cells will decrease the concentration of certain membrane bound calcium channels in order to preserve cell viability.[44] However, those same calcium channels contribute to the action potential plateau and their

decreased membrane concentration will decrease the action potential duration, which in turn reduces the refractory period and promotes the maintenance of AF by multiple circuit re-entry.[45, 46]

There are two competing models regarding the mechanism of re-entry. Allessie et al proposed the "leading circle hypothesis" which relies on the theory that there is a small circuit established with the smallest diameter possible that will maintain continuous activity.[41] This minimum circuit size is defined as the product of the refractory period and the conduction velocity. The core of the circle is continuously excited by impulses originating from the outer circle. Pertsov et al suggested the "spiral wave theory" in 1993.[47] This model depends on a fully excitable core with the maintenance of spiral wave re-entry dependant on the curvature of the wavefronts at the tip of the spiral. Each of these theories come with inherent limitations, highlighting the fact that although we have come a long way in understanding this complex arrhythmia, there is still work to be done.

1.4.2 Electrical remodelling – AF begets AF

The idea of tachycardia induced electrical remodelling was first suggested by two independent animal studies performed in the mid 1990's. In a dog model of chronic rapid atrial pacing, Morillo et al demonstrated a shortening of the atrial refractory period and more readily inducible sustained AF.[48] Wijffels et al developed a chronic goat model of sustained AF by attaching implanted electrodes to a fibrillation pacemaker that automatically delivered bursts of stimuli whenever sinus rhythm occurred.[49] By artificially maintaining AF, they showed even more marked refractory period shortening as well as increased inducibility and stability of AF. These observations led to the concept of "Atrial Fibrillation Begets Atrial Fibrillation", that tachycardia induced remodelling creates a substrate for persistent AF.[49] Although the results of these experiments opened a new avenue for researching and understanding AF, they failed to fully explain the phenomenon being witnessed. The changes of atrial refractory period demonstrated by Wijffels et al were near maximal after 24 hours, whereas the duration of AF continued to increase for 2 weeks.[49] In the years since the publication of these seminal papers

additional factors explaining tachycardia induced remodelling, including conduction velocity, wavelength and regional heterogeneity, have been proposed.[46] The basis of these changes have since been linked to ionic mechanisms, some of which are described above, most important of which is a reduction in L-type calcium channels.[50, 51]

1.4.3 Histological changes

The alterations in atrial tissue most frequently seen as consequences of AF are atrial fibrosis and loss of atrial muscle mass. It is important to mention that before anatomical changes occur, electrical remodelling is brought on by the initial AF stimulus. If AF is allowed to continue, anatomical remodelling will occur subsequently. Atrial fibrosis favours the development of re-entry circuits by creating physical barriers within the atria impeding the propagation of electrical activity. This causes a heterogeneous slowing of atrial conduction, as is the case in heart failure.[52] The renin-angiotensin-aldosterone system (RAAS) plays an important role in the development of atrial fibrosis. The RAAS is activated in response to atrial dilatation, with an increased expression of angiotensin converting enzyme within the atrial tissue. Angiotensin II and aldosterone facilitate the proliferation of fibroblasts and the deposition of proteins within the extracellular matrix, thus altering atrial structure.[53] Angiotensin receptor blockers and angiotensin converting enzyme inhibitors, medications frequently used for the control of blood pressure and in the treatment of heart failure, have been shown to delay or reduce the development of fibrosis and AF.[54, 55]

1.4.4 Autonomic influences

The development of AF can be secondary to changes in cardiac nervous tone as well. Coumel was one of the first to suggest that AF could result from increase in signalling of either of the components of the autonomic nervous system (ANS), i.e. sympathetic or parasympathetic.[56] He noted that when patients are asleep they can have AF episodes preceded by a sinus bradycardia, suggesting a vagal origin.[57] Conversely, it has been shown that AF can be associated with increased sympathetic

tone in the presence of intense emotional or physical stressors.[58] Coumel suggests that sympathetic AF occurs in patients with concomitant cardiac disease, while vagal AF in seen primarily in the absence of structural heart disease and generally yields paroxysmal AF.[58] The role of the ANS in AF is evidenced by the RR interval variation, corresponding to the distance between the two R waves (positive deflection of the QRS complex of ventricular depolarisation) on the electrocardiogram. This parameter has been shown to reliably identify sympathetic and parasympathetic influences on the heart, and variations recorded immediately prior to the onset of AF have made it possible to link these episodes to autonomic nervous system activity.[59, 60] The specific and relative contributions of each arm of the ANS remains to be determined, but the evidence is increasingly pointing to a synergistic effect between the sympathetic and parasympathetic systems.[61, 62] Changes in nervous tone in localized areas of the atrium create various zones with heterogeneous refractory periods. The result is zones of functional block favouring re-entry and, consequently, AF.[58] Hirose et al demonstrated that direct stimulation of the vagus nerves increases the heterogeneity of atrial refractory periods, thus facilitating induction of AF.[63] In a series of canine preparations, Jayachandran et al demonstrated that rapid AF, induced by rapid atrial pacing, results in a heterogeneous increase in atrial sympathetic innervation, with greatest heterogeneity in the right atrium.[64] Since the establishment of the role of the ANS in the pathophysiology of AF, further research has illuminated the importance of several anatomical sites as they relate to AF origin.

1.4.5 Anatomical origins of AF

The pulmonary veins

Various atrial regions have been reputed to play important roles in the initiation and maintenance of AF. The base of the pulmonary veins has emerged as a preferential site of micro and macro re-entry.[65] Haissaguerre was the first to identify this area as an important source of AF. While performing electrophysiological studies on a series of patients with drug refractory AF, he noted multiple ectopic atrial beats originating at the base of the pulmonary veins.[66] He demonstrated that

the origin of AF was found within a muscular band at the junction of the left atrium and the pulmonary veins.[67] The relatively chaotic organization of the area, including the orientation, sheathing, and ending of the muscular fibres, tends to favour the creation of local re-entrant circuits.[67-69] The embryologic origins of the pulmonary veins partially explain the aforementioned "chaotic" arrangement of these fibres. The base of the pulmonary veins represent the junction between the trunk of the pulmonary venous system, derived from the embryonic anterior intestine, and the left atrium, derived the primitive cardiac tube. The ionic characteristics of myocytes in this area also favour re-entry as a result of shorter refractory periods.[70, 71]

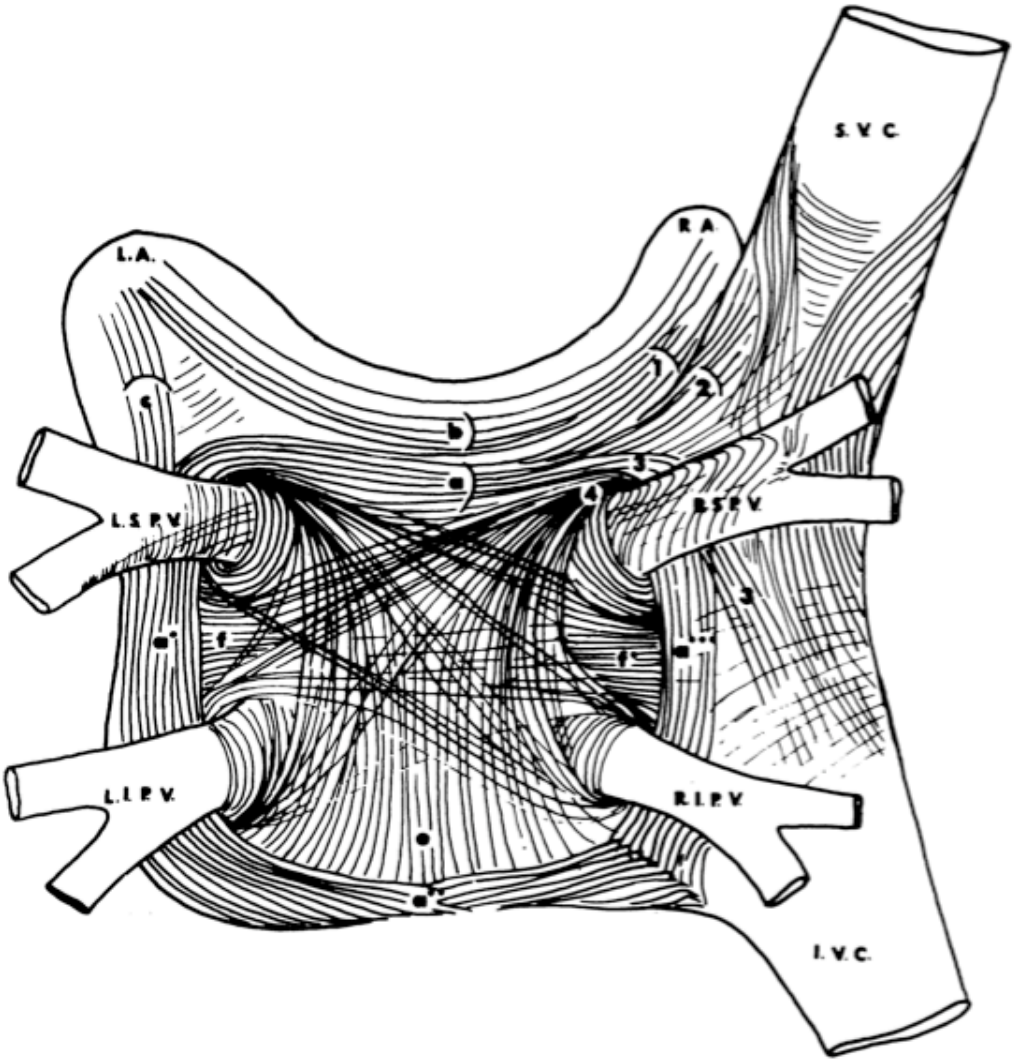

Figure 2. Schematic of superficial muscular fibres of the left atrium.

A main circular fascicle (a, a', a", and a"') runs peripherally around the area of the openings of the pulmonary veins, while an interatrial fascicle (b) runs between the right (RA) and the left (LA) atrium. Some fibres (c) descend from the left atrium into the left part (a') of the main circular fascicle. Circular fibres leaving the main fascicle turn around the openings of the pulmonary veins, forming sphincter-like structures; other fibres extend over the veins as myocardial sleeves. Loops of fibres coming from the atrium are seen over the right superior pulmonary vein (R.S.P.V.) and returning to the atrium. Oblique, vertical (e), and transverse (f, f') fascicles of fibres are also seen on the posterior atrial surface. L.A. = left atrium; R.A. = right atrium; S.V.C. = superior vena cava; I.V.C. = inferior vena cava; R.S.P.V. = right superior pulmonary vein; L.S.P.V. = left superior pulmonary vein; L.I.P.V. = left inferior pulmonary vein. (Reproduced from Nathan et al, Circulation 34(3):412-422, 1966).

Other sites of origin

There are several sites other than the pulmonary veins that have been identified as foci of AF initiation. In general, the junction between the atria and all major thoracic veins constitute possible sites of re-entry.[72] Although the left atrium is generally considered to be the more common site of AF initiation, the right atrium is also a potential source. Examples of the right atrium's arrhythmogenic potential have been seen following surgical or percutaneous interventions targeting only the pulmonary veins. Firstly, the onset of left atrial flutter can seen, possibly a result of incomplete ablation lines within the left atrium. Secondly, the elimination of left atrial AF mechanisms may allow for the emergence of right atrial sites that were otherwise overshadowed by their left atrial counterparts.[73] Forleo et al demonstrated that complex fractionated atrial electrograms were present in the right atrium and coronary sinus during pulmonary vein isolation procedures.[74] Even more interestingly, the authors were able to link these electrograms to an increased risk of AF recurrence. While there has previously been controversy regarding the right atrium's ability to initiate AF in the absence of left atrial sources, there is an increasing amount of evidence supporting this theory. When performing electrophysiological studies in 172 patients with AF, Chen et al identified sites of focal AF within the right atrium of 8 patients.[75] These sites were the crista terminalis (the right atrial free wall) and at the ostium of the coronary sinus. Similarly, Tsai et al demonstrated loci of ectopic beats at the SVC-right atrial junction that appeared to be sites of AF origin.[76] In both of these studies the authors were able to eliminate AF by radiofrequency ablation of these areas.

Interatrial conduction pathways comprise another group of atrial tissue with a tendency to initiate AF. Although any atrial tissue has the ability to propagate an electrical signal, conduction tends to occur via certain preferential tracts. The importance of Bachmann's bundle, also known as the interatrial band, as it relates to interatrial conduction was first described in 1916 by George Bachmann.[77] It is located at the upper extremity of the interatrial septum and the results of several studies have suggested its role in AF pathophysiology.[78-80] Other atrial structures studied in this context include the coronary sinus and fossa ovalis.[81] Finally, another

area of the atria that has been implicated in several studies as a site of AF origin is the ligament of Marshall. The ligament of Marshall is an atrial fold located adjacent to the coronary sinus in the inferior left atrium that is a remnant of the left horn of the sinus venosus in the foetus. It contains nerve fibres, muscle bundles, and the oblique auricular vein, the vein of Marshall.[82] The Marshall bundle is considered the most important interatrial conduction pathway in the inferior portion of the atria and is likely the site of AF origin in certain patients.[83, 84] Concordant with this assertion are the findings by Haissaguerre et al that the epicardial and endocardial regions bordering the coronary sinus are important in the maintenance of AF.[85, 86] Considering the above, it is clear that the sites of origin of AF are closely related to the anatomy and development of cardiac conduction tissue; consequently, a brief discussion of the embryologic development of cardiac conduction tissue will follow.

1.4.6 Embryologic development of the cardiac conduction system

Although the development of the cardiac conduction system remains controversial, one of the most accepted theories explaining the sequence of depolarisation of myocardial tissue is that of the integration of the neural crest during fetal development.[87, 88] Neural crest cells are said to participate in the formation of the four cardiac chambers and in the development of the cardiac conduction system.[88, 89] The ectomesenchymal neural crest cells migrate to the heart by routes along arterial and venous structures. The cells on the arterial side develop into the semilunar valves, while those on the venous side become integrated into the myocardium to form the atrio-ventricular node (AVN), the bundle of His, and the ventricular conduction branches.[90, 91] In addition, other authors have demonstrated a high concentration of cells surrounding the pulmonary veins.[92] In addition to its role in the morphogenesis of the cardiac chambers and the conduction system, the neural crest is implicated in the development of the intrinsic cardiac innervation.[93] This link between the nervous system and the heart is of utmost importance both in normal and pathologic cardiac function. There are a multitude of nervous fibres and ganglionated plexi on the surface of the heart that are important for the beat-to-beat

functioning of the heart.[94, 95] These autonomic nervous ganglia are preferentially distributed within epicardial adipose tissue at the base of the heart between the atria and ventricles, as well as around the great vessels. They have the ability to modify cardiac chronotropy, dromotropy, inotropy, lusitropy and bathmotropy.[96] The details of the cardiac autonomic innervation are in constant evolution but a review of our understanding to date is necessary in order to appreciate the findings of this work.

1.5 **The nervous system and the heart**

1.5.1 Neurocardiology

The ANS is responsible for maintaining homeostasis in the body. Classically, cardiac nervous control is said to result from a delicate balance between the two branches of the ANS, sympathetic and parasympathetic. Sympathetic influence is said to stimulate the heart, resulting in increased heart rate and force of contraction. While the parasympathetic, or vagal, influence has the opposite effect.[97]

The "classic" view of cardiac nervous control entailed the assumption of the heart's passive response to postganglionic sympathetic and parasympathetic signals, originating in the central nervous system (CNS).[98] However, although this concept of neuroanatomic organization held for over a century, it is now known that this not the entire picture. There exists a complex hierarchy of nervous structures that interact to influence cardiovascular function. The nervous structures that are implicated in this process are located at different levels of the body, notably in the brain (CNS), thoracic cage (extrinsic cardiac nervous system), and on the heart (intrinsic cardiac nervous system). This hierarchy is composed of many levels of feedback control loops that includes parasympathetic and sympathetic efferent and afferent neurons, intraganglionic neurons, and interganglionic neurons (Figure 3).[99] The notion of the heart's capacity for local integration and processing of neuronal signals was first put forth in the early 1970's by Armour and Ardell.[100, 101 102-104] It allows for the local integration and control of the many cardiac parameters that can be affected by the autonomic nervous system.[104-106]

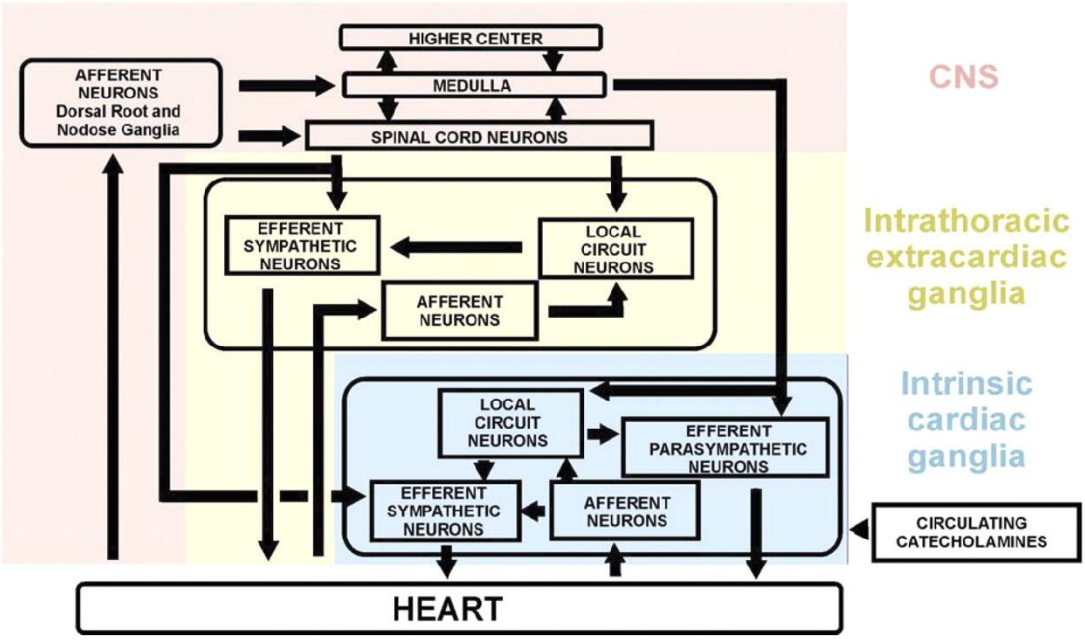

Figure 3 Proposed model for the cardiac neuronal hierarchy, emphasizing the intrathoracic components.[95]

1.5.2 The extrinsic cardiac nervous system

The thoracic cage is rich with both efferent (toward the heart) and afferent (toward the brain) nervous structures that are active in coordinating the nervous influences of the heart. This is the substrate of the extrinsic cardiac nervous system, with the efferent structures acting on the heart consisting of those descending from the CNS as well as the intrathoracic extracardiac ganglia. The coordination and local processing of these neuronal signals is mediated by local circuit neurons located within the intrathoracic ganglia.[105]

Transmission of information occurs between the CNS and the end-organ via a connection of two neurons in series, termed preganglionic and postganglionic. The preganglionic neurons develop from the neural tube and postganglionic from the neural crest. Preganglionic sympathetic neurons synapse in the paravertebral ganglionic chains. The principal sympathetic efferent ganglia innervating the heart are the stellate and median cervical ganglia. Parasympathetic innervation of the heart stems primarily from the nucleus ambiguus of the brain stem and is relayed to the heart through the vagus nerve, also called the vagosympathetic complex, with the

pre-post ganglionic synapse occurring at the level of the heart itself (Figure 4).[107-109] The axons exiting the nucleus ambiguus are composed of large myelinated fibres allowing for rapid transmission of neural signals with short latency between activation and the end organ effect of vagal bradycardia.[110] The principal parasympathetic afferent pathway, i.e. neuronal pathways leading away from the heart and toward the CNS, is the nodose ganglion, also known as the inferior ganglion of the vagus nerve. It has a cylindrical form and runs along the anterior aspect of the internal jugular vein. Cardiac sympathetic afferents reach the CNS via the dorsal root ganglia running along the spinal cord.[111]

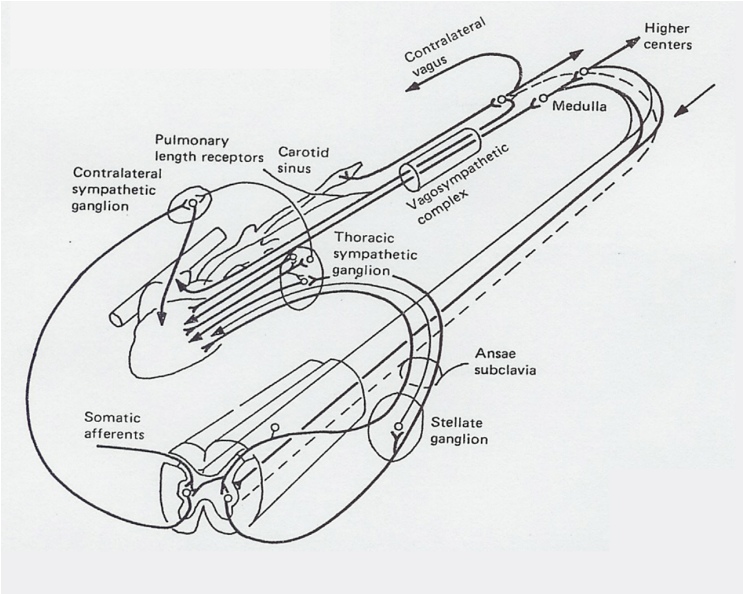

Figure 4 Schematic representation of the extrinsic cardiac nervous system at the level of the thoracic nerves.[109].

The local circuit neurons mentioned previously are a sub-population of neurons contained within the autonomic ganglia of both the intrathoracic extracardiac and intrinsic cardiac systems. They have projections to adjacent neurons within their own ganglion, or in other regional ganglia, allowing for a "functional interconnectivity" within the intrathoracic nervous system.[104, 112] The result is a capacity to influence cardiac performance in a manner that would not be possible with simple neuronal connections in series.

1.5.3 The intrinsic cardiac nervous system

The intrinsic cardiac nervous system (ICNS) is a complex network of neuronal fibres where sympathetic and parasympathetic nerves do more than simply deposit their signal. It is an extra level of neuronal control located directly on the heart's surface, otherwise known as the "little brain on the heart."[95, 96, 105] Neurons are located in the autonomic ganglionated plexi, which are clustered in discrete areas of the heart, primarily in the fat tissue on the epicardial surface of the heart. Four types of neurons can be found within these ganglia: efferent sympathetic and parasympathetic neurons, interneurons contained entirely within individual ganglion, local circuit neurons linking intracardiac ganglia, and sensory neurons mediating intracardiac reflexes.[113] The anatomical details of the atrial ganglionated plexi will be discussed below.

1.5.4 Anatomy of the intrinsic cardiac nervous system

The most complete account of the anatomy of ICNS was first described for the canine heart by Yuan et al in 1994.[114](Figure 5) Cardinal et al recently published a reproduction of Yuan's classification system.[115](Figure 6) Armour et al. used this work as a basis for their description of the human ICNS in 1997.[96] They analysed the hearts of 18 subjects and elaborated on the gross and microscopic anatomical details. They were able to consistently identify ganglionated plexi in five atrial and five ventricular locations (Figure 7)(Figure 8). Each ganglion contained anywhere from one to over 200 neurons. It is important to note that the precise location and size of each ganglion varied somewhat between specimens. They also noted connections between ganglia within each region forming "regional neural networks." The atrial ganglionated plexi as detailed by Yuan et al[114] are described below:

1) The **right atrial ganglionated plexus** (RAGP) is located in the adipose tissue just cephalad to the right atrial-IVC junction and just ventral to the interatrial groove. It has been shown that autonomic nervous signals preferentially, although not exclusively, pass through this plexus en route to the sinus node.[116]

2) The **left atrial ventral ganglionated plexus** is located on the ventral surface of the left atrium, it is comprised of three components: cranial, intermediate, and caudal. These were originally described in detail by Armour et al in 1990.[106]

3) The **dorsal atrial ganglionated plexus** is situated on the dorsal surface of the heart between the two atria.

4) **The inferior vena cava-inferior left atrium (IVC-ILA) ganglionated plexus** is located on the inferior dorsal aspect of both atria, adjacent to the entrance of the IVC into the right atrium. This plexus has been linked to the autonomic control of the AVN.[117]

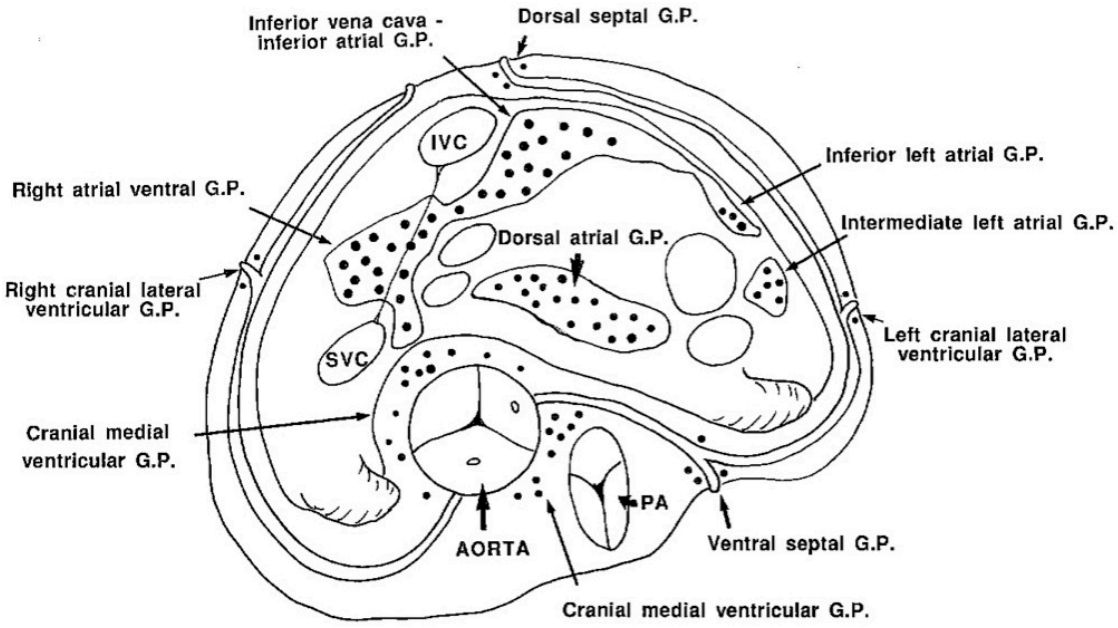

Figure 5. Distribution of ganglionated plexi on the canine heart, superior view.

GP = ganglionated plexus, PA = pulmonary artery, IVC = inferior vena cava, SVC = superior vena cava. [114]

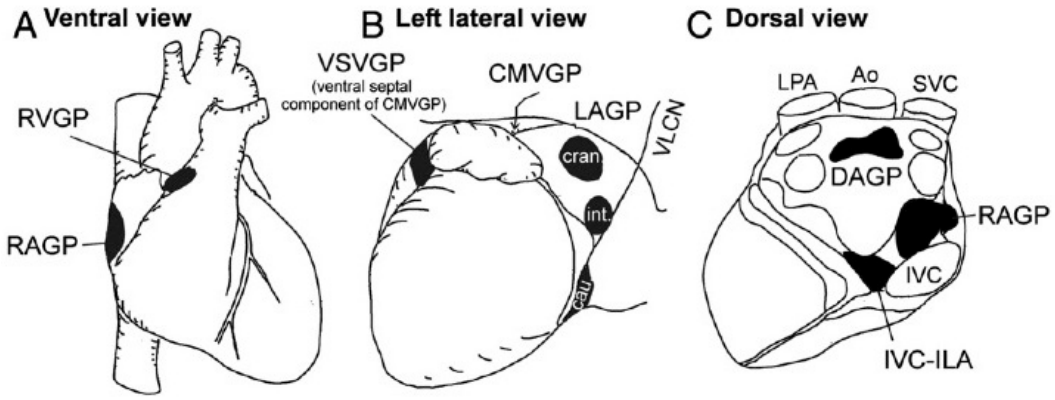

Figure 6. Anatomical locations of intrinsic cardiac ganglionated plexi in the canine heart.

A: ventral, B: left lateral, C: dorsal views of the heart. RAGP = right atrial ganglionated plexus (GP), RVGP = right ventricular GP, CMVGP = cranial medial ventricular GP, VSVGP= ventral septal ventricular GP, LAGP = left atrial GP, DAGP = dorsal atrial GP, IVC-ILA = inferior vena cava-inferior left atrial GP. VLCN=ventrolateral cardiac nerve, LPA=left pulmonary artery, Ao=aorta, IVC, SVC=inferior, superior vena cava. [115]

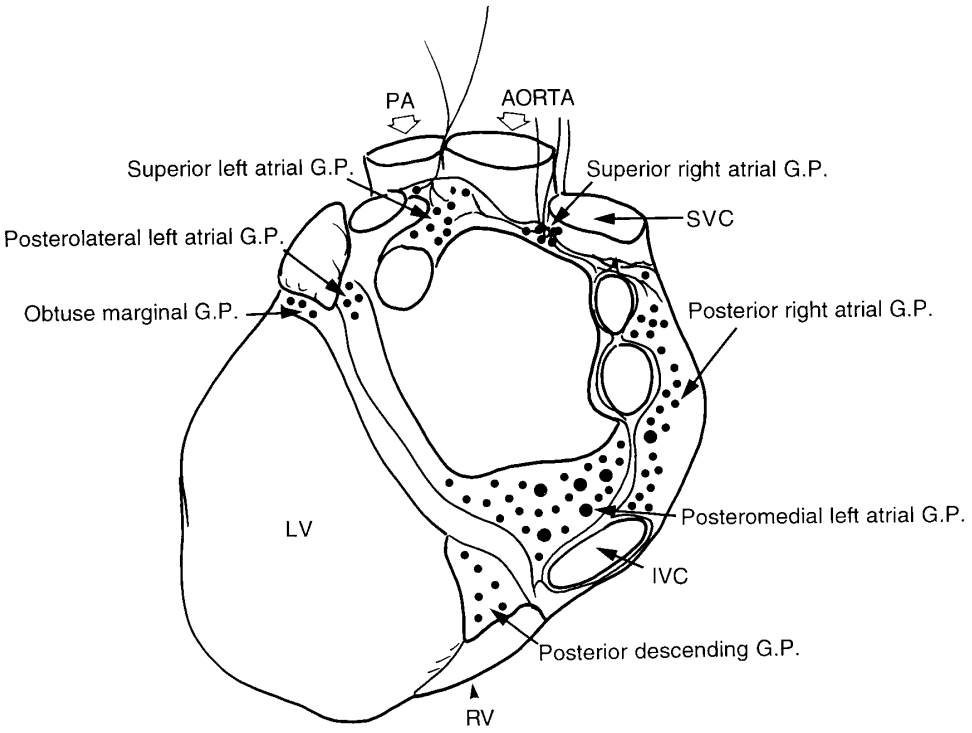

Figure 7. Distribution of ganglionated plexi on the human heart, posterior view.[96]

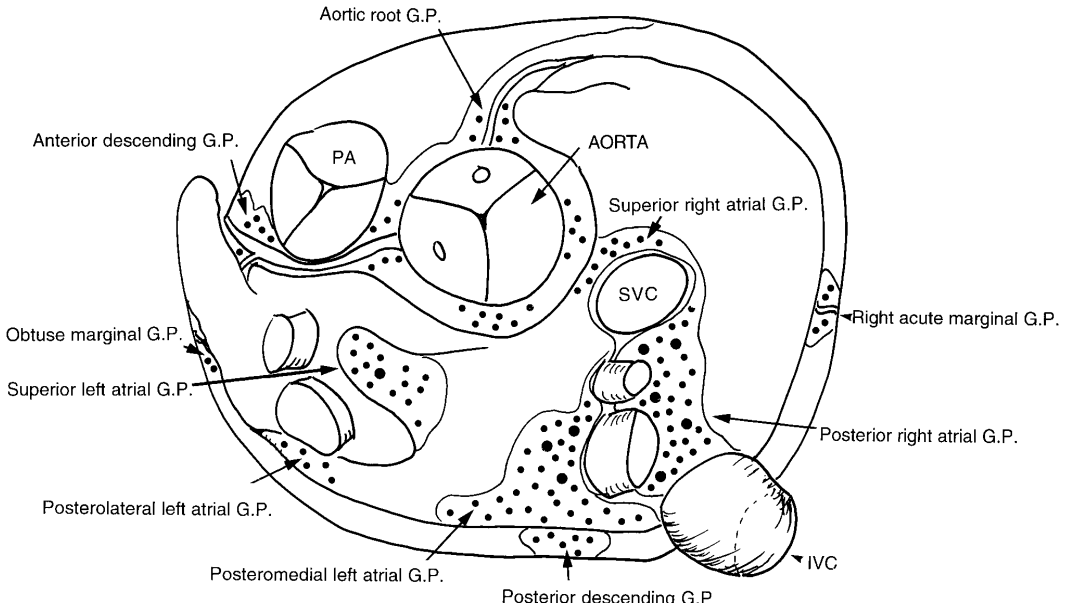

Figure 8. Distribution of the ganglionated plexi on the human heart, superior view.[96]

These ganglionated plexi were initially thought to be comprised of purely parasympathetic postganglionic efferents [117-119], without intrinsic neuronal activity and without accompanying sympathetic neurons. Both of these assumptions have since been refuted by demonstrating spontaneous activity of sympathetic and parasympathetic neurons within each plexus.[61]

1.6 **Management of Atrial Fibrillation**

The treatment of AF generally involves any or all of 3 objectives – the control of ventricular rate, prevention of thromboembolism, and restoration of sinus rhythm. The goals of treatment are tailored to each patient based on symptoms and comorbid conditions. Subclassification according to therapeutic modality can aid in the explanation of AF treatment options.

1.6.1 Pharmacologic treatment

Pharmacologic therapy is generally accepted as the first line of treatment in AF[5]. A variety of medications exist that are used to convert AF to sinus rhythm, control ventricular response, or both.[26, 120, 121] Accompanying their many benefits, these drugs can have significant side effects, including paradoxical proarrhythmic effects causing malignant arrhythmias or atrioventricular blockade as well as hypotension or exercise intolerance.[122, 123] Additionally, depending on the time to successful conversion to sinus rhythm, the risk of thromboembolism persists while on these medications. Anticoagulation therapy, along with its own substantial complication risks, must be maintained.[5, 124-126]

1.6.2 Invasive treatment

The suboptimal pharmacologic treatment options listed above have led to much effort focused on developing invasive techniques to control AF over the past two decades.[127-130] The objectives of these procedures are both to isolate arrhythmic foci and eliminate macro re-entry circuits. The maze procedure, described by Dr Jim Cox, is now considered the most effective treatment of AF.[129] The original procedure involved fragmentation of atrial tissue by the “cut and sew” technique. This entailed cutting apart the atrium at specific sites and suturing it back together in order to limit electrical continuity between certain areas. The resultant scar tissue barriers limit, in theory, macro re-entry circuits within the atria.

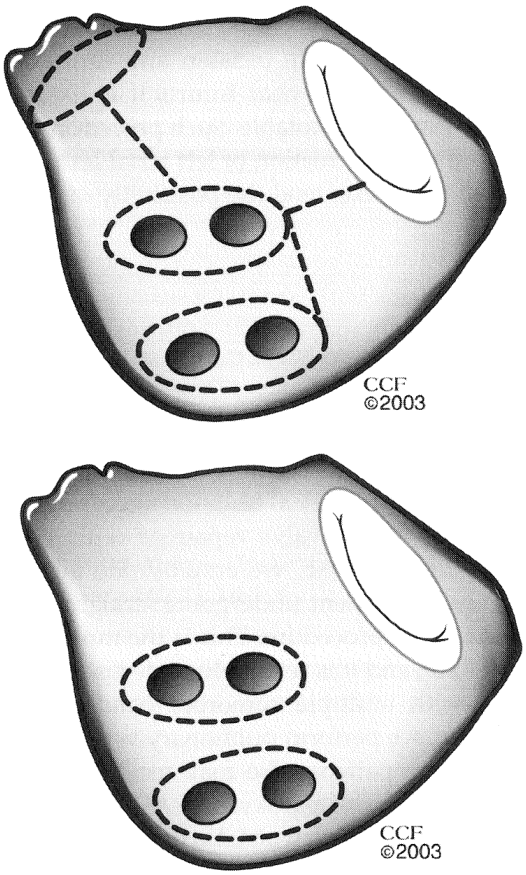

Figure 9. Ablation lines used in the invasive treatment of atrial fibrillation.

A – Left sided Maze procedure. B – Pulmonary vein isolation.[131]

More recently, the less invasive and faster procedures using radiofrequency, cryotherapy, and microwave technology, attempt to reproduce the full thickness tissue scarring of the original Maze procedure.(Figure 9)[132-135] In addition, percutaneous techniques have attempted to reproduce the results seen with surgery either by mapping the atria and directly ablating complexes that may be precursors to AF, or by targeting and isolating the trial tissue giving rise to AF.[136]

1.6.3 Invasive treatment of AF – Are we there yet?

Although the number of AF procedures has been rising consistently, there are still questions to be answered about their effectiveness. Many reports boast excellent

success rates, however the follow-up in some has been called into question. For example, periodic electrocardiograms and telephone interviews regarding symptoms may not present an accurate picture of AF recurrence. This weakness is highlighted by a study from Hindricks et al that demonstrated patients who were very symptomatic from their AF before a percutaneous intervention continued to have asymptomatic AF episodes following the procedure.[137] These episodes were found only with continuous electrocardiographic monitoring for 7 days using a portable monitoring device.

Regardless of how one might feel about the effectiveness of any given treatment, be it pharmacologic, percutaneous, or surgical, it is clear that the ideal treatment of atrial fibrillation is yet to be proven. The ideal treatment would be minimally invasive, very effective, and eliminate the negative consequences of AF, most notably symptoms and the need for anticoagulation. Pharmacologic treatment is incapable of returning many patients to sinus rhythm and when it does it is often at a price of significant complications, including malignant arrhythmias, severe side effects, and bleeding.[138] Percutaneous and surgical treatment have improved dramatically with few complications, however success rates are variable and reporting of results are inconsistent, as mentioned above. As a result of the abovementioned shortcomings, research in this area has become very active in recent years. One of the most active areas of study is the intrinsic cardiac nervous system and it's associated autonomic ganglia.

1.6.4 The ICNS and control of cardiac functions

As discussed earlier, the ICNS is both the intermediary between the ANS and the heart, as well as an independent entity that contributes to beat-to-beat variations of cardiac activity.[104] Intrathoracic nervous elements respond to intrinsic stimuli as well as extrinsic electrical stimulation, thus raising the possibility of influencing the electrophysiological and dynamic properties of the heart.[115] Furthermore, Chiou et al demonstrated that selective denervation was possible when radiofrequency ablation was applied to epicardial ganglionated plexi. They were able to modify the atrial response to vagal stimulation by ablating three areas of cardiac ganglionated plexi

without affecting the ventricular response to vagal stimulation.[139]. Other studies using dog models have clearly demonstrated that electrical or chemical stimulation of various nervous elements can modify cardiac activity.[95, 102, 105, 106, 115, 140, 141] Furthermore, it has been shown that there is considerable redundancy in the cardiac innervation, revealing the complexity of the cardiac nervous system.[142] The idea of modifying cardiac arrhythmias by stimulation and ablation of atrial tissues has been exploited in percutaneous and surgical AF interventions.[143-146] In summary, the ICNS is a potential therapeutic target for modulation of cardiac activity.

1.6.5 The ICNS as it relates to AF

As discussed earlier, the pathophysiology of AF involves structural, electrical, and neurophysiologic elements. The majority of pharmacologic and invasive therapies for AF target the former two elements. The importance of neurophysiology, particularly the ICNS, in AF pathogenesis and control is becoming more and more evident. In an animal model, Scherlag et al were able to induce AF by simulating ectopic firing from the pulmonary veins only if the adjacent ganglionated plexi were stimulated simultaneously.[147] Huang et al induced ventricular arrhythmias with stimulation of intrinsic cardiac neurons in a dog model.[148] Several other experimental models have implicated ganglionated plexi and the ICNS in the initiation of both atrial and ventricular arrhythmias.[149, 150] These findings have encouraged researchers and clinicians to target the ICNS in novel therapies to control or eliminate AF.

1.6.6 Neuromodulation by direct ablation to control atrial arrhythmia.

There have been an overwhelming number of reports in recent years describing results following invasive treatment of AF. Many authors have suggested that much success is due to the phenomenon of neuromodulation.[82, 136] Pappone et al reported on 297 patients who had underwent circumferential pulmonary vein ablation, with 34% of these patients having a bradycardic response to stimulation. This subgroup of patients seemed to have a better response to ablation with 99%

freedom from AF at 12 months compared to 85% in the group not presenting a bradycardic response to stimulation. [151] Mehall et al reported a minimally invasive approach for atrial fibrillation treatment which included localization of epicardic ganglionated plexi by electrical stimulation followed by radiofrequency ablation.[152] Like Pappone, the authors presumed that by localising and ablating ganglionated plexi they had eliminated additional arrhythmogenic foci. They propose this to be a form of vagal or autonomic denervation and suggest that these should become a routine addition to ablative treatment of AF.

Pappone's observations led to increased interest in exploring AF treatment by direct neuromodulation through denervation. Scherlag et al described a canine model in which ganglionated plexus stimulation was thought to enable AF induction when performed concomitantly with pulmonary vein stimulation.[153] Other authors have since reported similar experimental results, however, the causal link between ganglionated plexi and AF has yet to be established.[154] Schauerte showed that direct stimulation of atrial ganglionated plexi led to a shortening of the atrial refractory period that facilitated AF.[155] They also showed that ablation of those plexi reduced the inducibility of AF through vagus nerve stimulation. Observations of this nature gave researchers the physiologic basis to modulate atrial arrhythmias through focal epicardial ablation. Other authors have reported conflicting results. Hirose, among others, found that partial denervation of the right atrium actually increased AF susceptibility.[63, 156, 157] These observations potentially demonstrate not only the relationship between the autonomic nervous system and cardiac arrhythmias, but also the possibility to influence atrial arrhythmias through neuromodulation. The fact remains that, despite much research, the hypothesis of selective autonomic denervation to treat AF has not yet been proven. All studies relating AF to the ANS have been through indirect evaluation, relying mostly on heart rate variability to implicate ganglionated plexi in experimental or clinical result.

1.6.7 Direct neuromodulation: Many unanswered questions

An ill-defined target

Although there is some clinical evidence supporting the use of cardiac denervation in the treatment of AF, the technique has significant hurdles to overcome. Work by Tan et al suggests that the idea of selective neuromodulation employed during endovascular and surgical procedures is at best optimistic, since each ganglionic bundle contains both sympathetic and parasympathetic neurons.[61] This theoretical problem can be reconciled by considering data from Patterson et al who demonstrated in experiments with isolated canine pulmonary veins that concomitant simulation of parasympathetic and sympathetic neurons may be more effective in induction of atrial ectopy. This suggests that it may in fact be favourable to target both types of autonomic influence together. In a human study, Cummings et al found that resection of the anterior aortic fat pad actually increased the incidence of postoperative AF.[156] Finally, although it may be clear that autonomic nervous elements are in some way implicated in AF, it is still unclear what these elements are, where they can be found, at what level of the ANS they must be targeted, intrathoracic versus the ICNS for example, and by what method they should be approached.

Durability of denervation?

The uncertainties of denervation procedures go beyond selecting the target. Even if the arrhythmogenic ganglia were clearly identified, there is significant evidence suggesting that effects of denervation on cardiac tissue are only transient.[158, 159] In a canine model, Murphy et al found evidence of nerves crossing suture lines one year following cardiac transplantation.[160] Evidence of sympathetic re-innervation has been described in the clinical transplant literature as well, with growth that occurs even up to 15 years following transplantation.[161, 162] Even Pappone et al, in their seminal paper on pulmonary vein denervation, remarked that heart rate changes associated with vagal stimulation returned to baseline levels at 6 months, a sign of autonomic re-innervation.[151] In an experimental model, Oh et al demonstrated that autonomic nervous changes induced by epicardial ganglionated

plexus ablation reverted to normal after 6 weeks.[158] Therefore, it would seem that cardiac re-innervation might limit the durability of direct neuromodulation.

A third issue that may limit the applicability of direct neuromodulation in AF treatment is the paradoxical reaction that occurs following intervention termed "nerve sprouting".[156] Okuyama et al showed that radiofrequency ablation in a dog model produced a hyperinnervation of cardiac tissue rather than denervation.[163] Chen et al showed that nerve sprouting following myocardial injury results in an electrophysiological heterogeneity that can increase the incidence of ventricular tachyarrhythmia.[164]

In summary, while direct neuromodulation may play a role in the treatment of AF, the ideal method has not yet been described. Furthermore, although some have reported clinical success using the bradycardic response as a road map for ganglionated plexus ablation, the neurophysiologic and anatomical basis for their success, or failure in some cases, is certainly not clear. Although advances in science, and medicine in particular, are often stumbled upon by chance, it is of utmost importance to explore the fundamentals of these advances in order to better understand why or how it has occurred and to help guide clinical practice and future development.

1.7 **Hypothesis**

As described above, the intrinsic cardiac nervous system is comprised of several ganglionated plexi located in areas of adipose tissue dispersed over the epicardial surface of the mammalian heart. According to Yuan et al's classification, there exist 4 atrial ganglionated plexi on the canine heart.[114] (Figure 6) It has been well established that atrial ganglionated plexi assert some control over various cardiac functions including, among other things, chronotropy. However, the anatomic pathways followed by these ganglionic nerve fibres have not been explored or characterized in any detail. Despite this, there are many authors who, during ablative interventions for AF perform electrical stimulation of these areas, and upon witnessing a bradycardic response, proceed to ablation of the area (See

sections 1.6.6 and 1.6.7). Although some publications report positive outcomes, the results are far from conclusive, leaving unresolved questions regarding the reproducibility of this technique and the overall arrhythmogenicity of the LAGP neurons. Understanding these pathways may play an important role in determining the ideal interventions, if any, targeting the intrinsic cardiac nervous system in the treatment of atrial fibrillation.

When considering the anatomic locations of each of the 4 atrial ganglionated plexi as they relate to the bradycardic response seen with epicardial stimulation, some obvious questions arise. The right atrial ganglionated plexus (RAGP) is in close proximity to the sinus node, making it easy to understand the short distance nervous structures must travel in order to arrive at the sinus node and effect changes in cardiac chronotropy. The left atrial ganglionated plexus (LAGP), however, is located lateral to the left pulmonary veins and anatomically distant from the sinus node. The physioanatomic pathways of the bradycardic response seen with stimulation of the LAGP are appreciably more difficult to comprehend.

There exist three possible neuroanatomic pathways that could explain the mechanism of the sinus bradycardia witnessed with stimulation of the LAGP. 1) A central reflex arc via the vagus nerve that travels from the LAGP, to the central nervous system and then back down to the right sided heart via the opposite vagus nerve. The bradycardic effect of the vagus nerve is beyond question and the neural and arrhythmogenic consequences of vagal stimulation have also been well established.[141] 2) A pathway through the pericardiac plexi, namely the periaortic or superior vena caval plexi. Mediastinal neuronal projections have been found travelling along the superior vena cava and have been associated with arrhythmia induction.[165] The peri-aortic plexi have been less thoroughly studied but may also play some role in arrhythmia formation[96, 156, 165]. 3) A direct intracardiac pathway that has not yet been described may play a role in LAGP-sinus node interactions. Finally, each of the above pathways may contribute in some capacity.

In addition, although we presume that the LAGP is responsible for the electrophysiological changes seen with direct epicardial ganglia stimulation, the

regional distribution of the response has not yet been characterized. It remains unknown whether neuronal activation occurs locally, across the entire atrium, or remotely from the stimulation site.

We hypothesise that the LAGP communicates with the sinus node via a central pathway along the vagus nerves and that repolarization changes occur in both the right and left atrium with LAGP stimulation.

1.8 **Objectives**

The objective of this work was to delineate the mechanism of the bradycardia induced by stimulation of the LAGP. To accomplish this, in addition to identifying the pathways responsible for the bradycardic response, we aimed to map the pattern of atrial repolarization changes following LAGP stimulation.

Chapter 2. Methods

Twenty-two adult mongrel dogs of either sex, weighing 25-35kg, were studied during this experimental protocol. LAGP stimulation was performed at baseline and then following each ablation sequence.

The pathways responsible for the bradycardic response were identified through sequential ablation of known autonomic pathways, including vagus nerve sectioning, radiofrequency ablation of periaortic and vena cava ganglionated plexi, and ablation of the RAGP, which is responsible for most autonomic input into the sinus node. Both antegrade and retrograde ablation sequences were performed (see below for details). Epicardial isointegral mapping was performed to accomplish the further goal of characterizing atrial repolarization changes following LAGP stimulation.

2.1 Ethics

Experiments were performed in accordance with guidelines set out by the Canadian Council of Animal Care[166] and approval was obtained from the animal research ethics committee of the Sacré-Coeur Hospital Research Center.

2.2 Experimental protocol

2.2.1 Anaesthesia

Anaesthetic induction was performed using intravenous sodium thiopental (25 mg/kg) followed by hourly boluses of alpha-chloralose (25mg/kg) intravenously. Alpha chloralose was chosen because it is thought to have a limited effect on neuronal activity. Each bolus was administered over a period of 5 minutes to minimize hemodynamic disturbances. Depth of anaesthesia was evaluated continuously by monitoring of jaw tension and by variations in heart rate and blood pressure. Mechanical ventilation was maintained with a Harvard Apparatus (Millis, MA). Blood oxygen saturation was monitored continuously with lingual pulse-oxymetry (VetOx G2 Digital, Dolphin medical, Hawthorne, CA). Oxygen flow was

adjusted to maintain a partial oxygen saturation (SpO_2) of 95% or greater. Body temperature was maintained using a heated mat and infrared lamp. The left femoral artery and vein were cannulated to facilitate invasive blood pressure monitoring and fluid administration.

2.2.2 Surgery

Bilateral anterior thoracotomy was performed with the aid of electrocautery to ensure adequate hemostasis and both internal mammary arteries and veins were ligated with braided silk sutures. The animal is paralyzed (rocuronium 0.5mg/kg) during this time to facilitate the initial dissection. A pericardial cradle is created by a vertical midline pericardial incision and fixation to the lateral chest walls. This ensures exposure of the ascending aorta, superior vena cava, right and left atrial ganglionated plexi, and the entire heart. The vagus nerves are exposed in the cervical region and encircled with umbilical tape to aid in instrumentation. Epicardial electrodes were placed on the atrial and ventricular surfaces for monitoring and pacing, respectively. In order to facilitate data analysis, atrial electrograms were isolated by atrioventricular (AV) nodal blockade with formaldehyde injection (37%, 0.1-0.2ml) into the AV node. Right ventricular pacing (60bpm) was instituted to maintain adequate cardiac output between periods of nerve stimulation.

2.2.3 Neuronal stimulation

Stimulation of both vagus nerves, the RAGP, and the LAGP was performed at baseline and following each ablation sequence.

Vagus nerve stimulation

Two unipolar electrodes are installed on the vagus nerves bilaterally. These electrodes consist of two metallic wires (Medwire, Mount Vernon, NY), individually mounted on 25 gauge needles and inserted 1cm longitudinally into the vagus nerve. Electrical stimulus is transmitted via a programmable stimulator (Bloom Associates, Philadelphia, PA). A supramaximal stimulation (15 Hz, 1mA, 1ms) is transmitted to

the vagus nerves. Electrical current is derived from batteries and controlled by a grass SD9 stimulator. Amplitude is adjusted according to cardiac response.

Ganglionated plexus stimulation

Stimulation of the RAGP and LAGP was accomplished by application of bipolar electrodes (1.5 mm apart) mounted on a probe connected to a battery-driven current source controlled by a programmable stimulator (Bloom Associates, Philadelphia, Pa). Stimulation was triggered by a reference signal derived from a pair of electrodes sutured onto atrial muscle. A train of 5 electrical stimuli (15Hz, 3-4mA, 1ms) was delivered to the RAGP and the superior, intermediate, and inferior left atrial epicardial ganglionated plexi during the atrial refractory period to avoid muscle activation. Stimulation was continued until the bradycardic response stabilized or it became clear that no bradycardia would occur at a given site. This was performed at multiple sites in each left atrial ganglionated plexus region to ensure identification of a bradycardic response if the potential existed. If multiple sites in the LAGP responded to stimulation (e.g. cranial and caudal plexi), the area with the greatest bradycardic change was used for data analysis.

2.2.4 Neuronal ablation

Vagal decentralisation

As the only direct pathway of communication between the ICNS and the central nervous system is the vagus nerve, this was chosen at the target for decentralisation. Decentralisation was accomplished by vagus nerve sectioning at the mid cervical level.

Ganglionated plexus ablation

Ablation of the periaortic, SVC plexi, and the RAGP was accomplished with a monopolar radiofrequency probe (Medtronic, Minneapolis, MN). The probe was set at 25 Watts, with ablation times of 20 seconds per centimetre to ensure transmurality. Periaortic ablation consisted of RF application to all epiaortic fat surrounding the aortic root. SVC plexus ablation consisted of circumferential RF

application to the SVC and the fatty tissue located medially. The RAGP was ablated by RF application to the entire collection of adipose tissue located on the right atrium just below the SVC-right atrial junction.

2.3 **Electrocardiographic evaluation**

2.3.1 Activation Mapping

Silicone plaques carrying 191 unipolar recording contacts (4.6- to 5.9-mm spacing) were positioned on right and left atrial surfaces in order to capture atrial unipolar electrograms.[141, 142] These plaques covered the right atrial free wall, inferoposterior left atrium and coronary sinus, the posterior left atrium between the pulmonary veins, the left atrial free wall, and the interatrial band (Figure 10). A surface electrogram is recorded at each electrode and the signal is transmitted to a multichannel recorder (EDI 12/256, Institut de génie biomédical, École Polytechnique de Montréal) controlled by custom-made software (Cardiomap III: www.crhsc.mtl.rtss.qc.ca/ cardiomap) using a PC computer. Unipolar electrograms (measured with reference to 4 limb leads) were amplified (0.05 to 450 Hz) and converted to digital format at 1000 samples/s/channel. Activation times were identified at maximum negative potential displacement (dV/dtmax). Colour-coded isochronal maps (10-ms intervals) were computed automatically by linear interpolation. The site corresponding to the earliest activation is determined at baseline in sinus rhythm and with the earliest bradycardic beat during neuronal stimulation (Figure 11).

2.3.2 Repolarization mapping

The spatial distribution of neural effects on repolarization was assessed using isointegral mapping. The Cardiomap program was used to adjust the baseline joining isoelectric segments between the end of repolarisation of one atrial activation complex and the beginning of the following activation. The net area (integral) subtended by each unipolar electrogram is calculated by the modified Simpson method.[167] This is measured at each recording site during baseline sinus beats and

sinus beats during LAGP stimulation. By algebraic subtraction of the integral value for the basal beat from the integral value measured during nerve stimulation, area difference maps are plotted indicating the atrial regions that were affected by nerve stimulation.[141, 142](Figure 12) Regional atrial repolarization maps can be presented as either selected examples in individual experiments or cumulative maps summarizing data from several animals. Cumulative incidence maps indicate, for each recording site, the proportion of animals in which repolarization changes were induced beyond a threshold of 30mV·ms (corresponding to the maximal variation among unipolar recordings of 2 standard deviations of changes in repeated measurements under baseline conditions). A typical example of right vagus nerve stimulation is shown in Figure 13 with the blue areas representing positive isointegral changes, which are repolarisation changes seen on atrial electrograms.

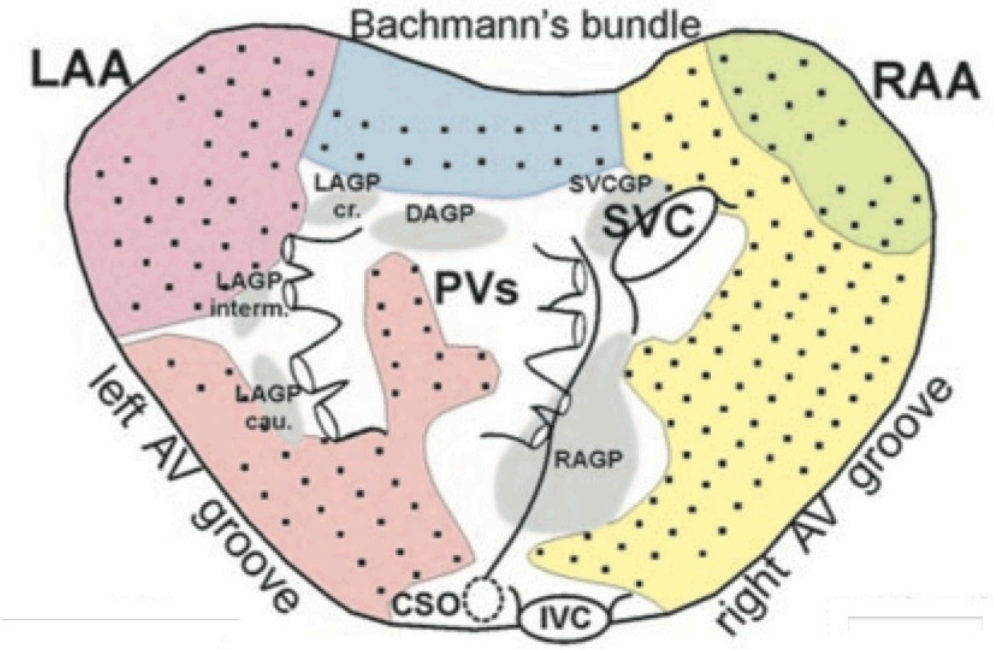

Figure 10. Schematic representation of atrial epicardial mapping electrode plaques.

The left atrial (LA) and right atrial (RA) surfaces are shown unfolded and viewed from a posterior projection. Epicardial plaque electrodes were positioned over 1) the RA free wall (yellow) and appendage (green), (2) the lateral LA wall and posterior LA wall between the pulmonary veins (PV) (orange), (3) the LA appendage (LAA) (pink), and (4) Bachmann's bundle (blue). SVC = Superior vena cava, IVC = Inferior vena cava, CS = Coronary sinus.

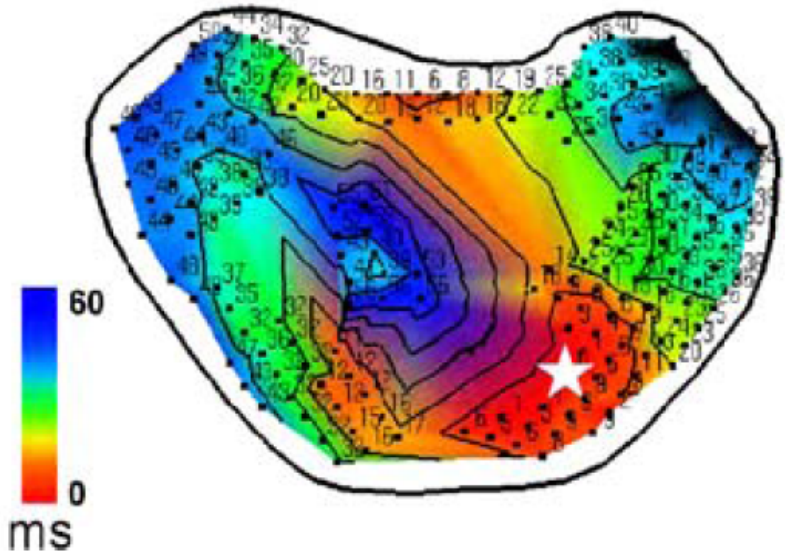

Figure 11. Example of atrial activation map.

The star represents the site of earliest atrial activation. The colours represent the time elapsed between the site of earliest activation and all other recording sites.

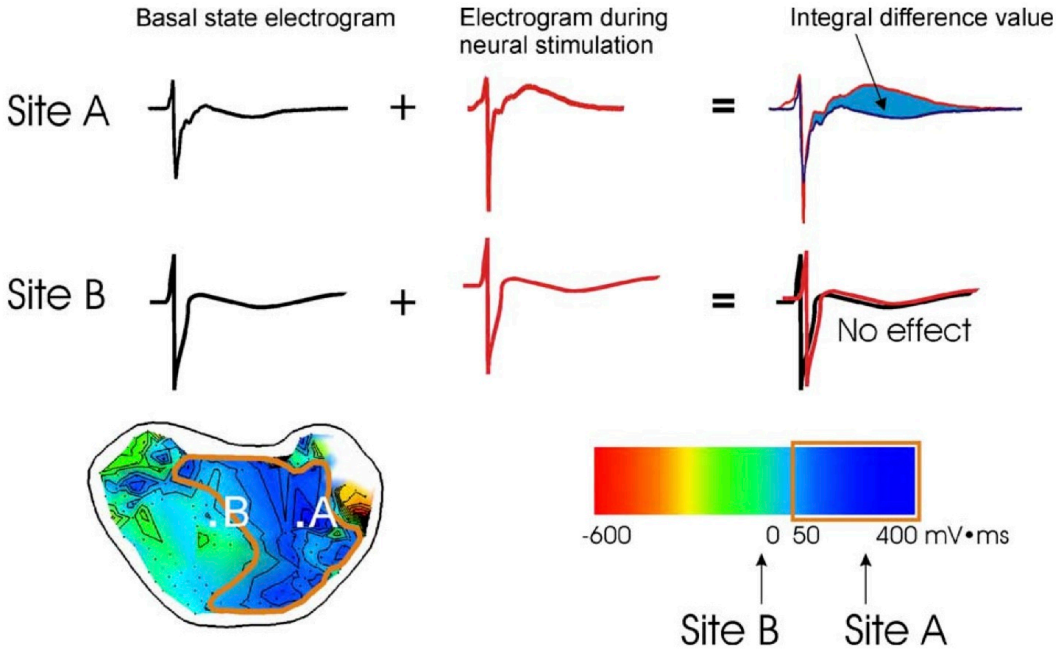

Figure 12. Principles of isointegral mapping.[141]

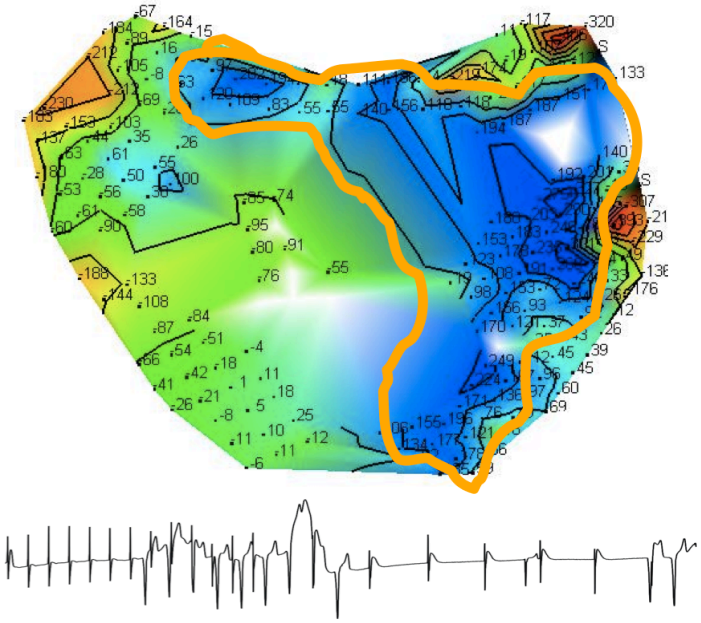

Figure 13. Example of repolarisation map during vagal stimulation.

The colours represent changes in the repolarisation period. The blue area (delineated by the orange line) corresponds to the zone where all changes were significant (greater than 50mV·ms) when comparing stimulation to the Baseline state.

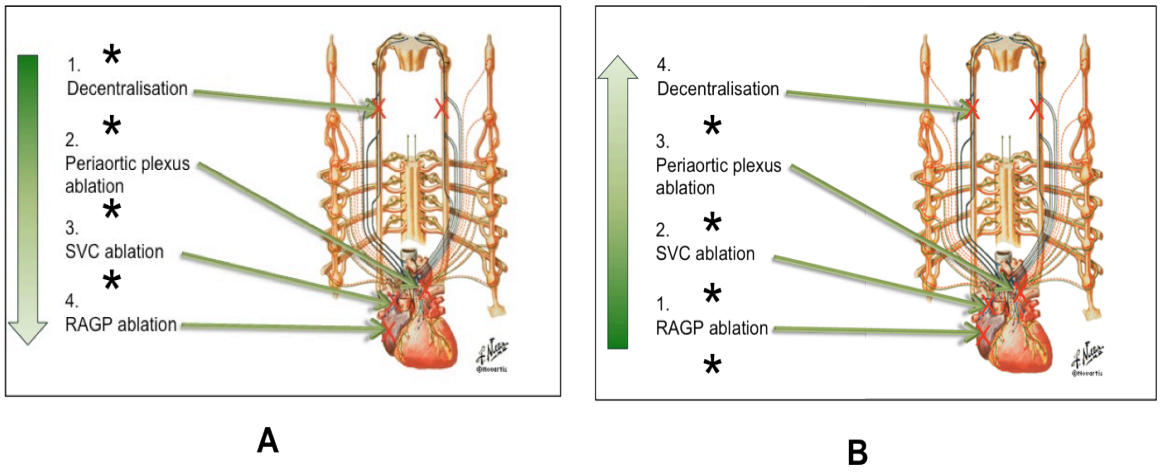

Figure 14. Experimental protocol.

A – Antegrade ablation sequence, B – Retrograde ablation sequence. * Denotes LAGP stimulation with activation and repolarisation map acquisition. (Modified from Netter, Atlas of Human anatomy, 2nd edition, 1997)

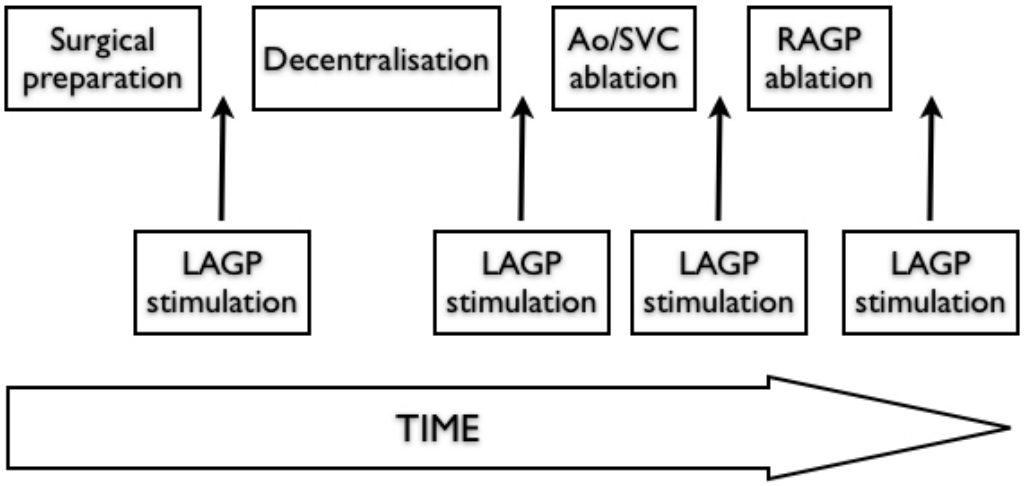

Figure 15. Experimental protocol timeline.

Sequence of stimulation and ablation for the antegrade group. The sequence was reversed in the retrograde group.

2.4 **Experimental protocol**

The experimental protocol is depicted in Figure 14 and Figure 15. Following surgical preparation, as described above, baseline activation maps are recorded. This is followed by sequential stimulation of the right vagus nerve, the left vagus nerve, the RAGP, and the cranial, intermediate, and caudal LAGP. The atrial response to each stimulation is recorded with the help of the 191 electrodes on the epicardial surface. This permits the establishment of baseline values of the bradycardic response to vagal and plexus stimulation. Stimulation of these structures is carried out following each ablative intervention, as shown in Figure 14. The protocol included both an antegrade and a retrograde ablation sequence, in order to control for time related variations in neuronal response. In the antegrade group (n=17), the ablation sequence was: 1) Decentralisation, by vagal sectioning, 2) Radiofrequency ablation of the peri-aortic and SVC plexi, 3) Radiofrequency ablation of the RAGP. At baseline and following ablation of each pathway, stimulation of both vagus nerves, the RAGP, and the cranial, intermediate, and caudal LAGP is performed sequentially. If there was no inducible bradycardic response to LAGP stimulation either at baseline or after a given ablation set, the experiment was continued throughout the subsequent ablation step. This was done to maximize the possibility

of eliciting a bradycardic response from the LAGP if at all possible. In the retrograde group (n=5), ablation was performed in the following order: 1) RAGP radiofrequency ablation, 2) peri-aortic SVC radiofrequency ablation, 3) decentralisation by bilateral vagus nerve sectioning. Vagus nerve and ganglionated plexus ablation was performed as described above for the antegrade group.

In order to limit confounding factors related to hemodynamic changes during induced bradycardia, ventricular pacing wires were placed on the right ventricle at initial preparation. The ventricles were paced at 60bpm throughout the experiment, maintaining adequate arterial pressure and cardiac output. Ventricular pacing was only briefly interrupted during neuronal stimulation to allow data acquisition without artefact from ventricular activation.

2.5 **Data analysis**

Parameters studied were atrial bradycardia, spatial distribution of effect of LAGP stimulation on atrial tissue, and the effect of LAGP stimulation on bradycardia and atrial repolarisation with each ablation sequence.

2.5.1 Bradycardia

Subtracting the time interval between each atrial beat at the sinus node allows one to determine the sinus rate at baseline and during stimulation. The difference between the atrial rate at baseline and during stimulation defines the bradycardic effect of that stimulation. This difference was calculated at baseline and then compared to subsequent stimulations following each step in the ablative protocol.

2.5.2 Isointegral distribution mapping following neuronal stimulation

The incidence of regional neuronal repolarisation effects was assessed by tallying, for each recording site, the number of preparations that represented changes on the integral electrogram greater than a threshold value of +30 mV·ms or the equivalent of 2 standard deviations of the value obtained

by subtracting two sets of measurements recorded during basal conditions.[141] The results are represented graphically on an atrial map depicting the position of the 191 electrodes. Cumulative incidence maps were generated, with the number of preparations that showed significant repolarisation changes (REPOLΔ) at each site indicated by the designated colour code. The atrial surface (mm^2) showing REPOLΔ was calculated based on the number of electrodes recording changes above the pre-established threshold. This calculation takes into account the triangular area formed by three recording electrodes, which varies from $0.22cm^2$ to $0.35cm^2$. To facilitate data analysis and interpretation, the electrode plaques were divided into the following six atrial regions (see Figure 16); 1) right atrial free wall, 2) right atrial appendage, 3) Bachmann's bundle, 4) left atrial appendage, 5) left atrial free wall, 6) posterior left atrial wall (between the pulmonary veins).

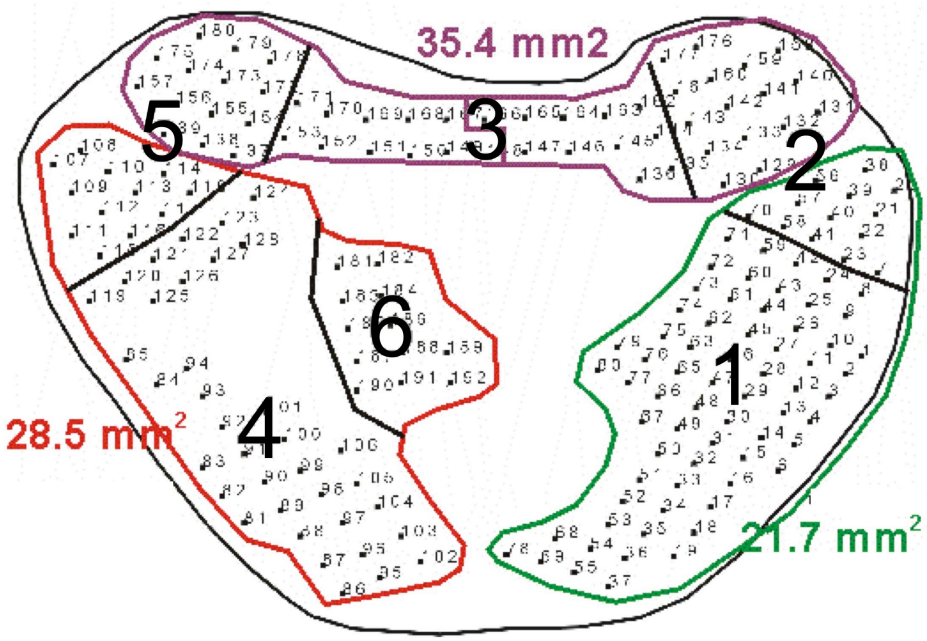

Figure 16. Designated atrial regions based on electrode plaque location.

1) Right atrial free wall, 2) right atrial appendage, 3) Bachmann's bundle, 4) left atrial appendage, 5) left atrial free wall, 6) posterior left atrial wall (between the pulmonary veins). Coloured numbers on edge of map represent area enclosed by a triangle between electrodes on a given plaque.

2.5.3 Effect of LAGP stimulation atrial repolarisation with sequential ablation

The changing effects of LAGP stimulation on atrial bradycardic response and atrial repolarisation were assessed. For each preparation, the bradycardic response at baseline was compared to the response following each subsequent ablation set. Surface area of atrial repolarisation change were analysed in the same way. The results for each preparation were analysed together to generate cumulative incidence maps.

2.5.4 Statistics

The data was analysed using the statistics software SPSS version 16. The paired Student t test was used to compare continuous variables, i.e. bradycardic response to stimulation and the distribution of neuronal influence on atrial repolarisation. Statistical significance was set at 0.05. The data is presented as the mean ± standard deviation when appropriate.

Chapter 3. Results

3.1 Bradycardia

3.1.1 LAGP stimulation, antegrade group

Bradycardia was induced in 17 of 22 animals, equivalent to 77%. This included 12 of 17 dogs in the antegrade group and 5 of 5 in the retrograde group. This is represented graphically in Figure 17. A persistent bradycardic response following RAGP ablation was present in 5 of 12 dogs when both groups are combined. Figure 18 shows the average cycle length change with LAGP stimulation in the antegrade group at baseline and following each step in the ablation protocol. In this figure, all preparations were included for analysis, regardless of bradycardic response to stimulation following ablation. In basal conditions, the cycle length (CL) increased by 38±15 ms ($p<0.002$). This corresponds to a slowing of the sinus rate from 149±34bpm to 136±28bpm. Figure 19 compares the average change in cycle length from baseline and following ablation at each step of the antegrade protocol. Values were only included for analysis if the bradycardia was induced at baseline and following a given ablation. There was no significant change in cycle length following decentralization by vagus nerve sectioning. Following periaortic SVC ablation, cycle length change decreased significantly, from 44.7±11.6 to 26.1±14.3 ms. There was no significant difference in cycle length change in the two animals that maintained a bradycardic response with RAGP stimulation.

In the retrograde ablation group, there was a significant lengthening of cycle length with LAGP stimulation at baseline in all canines, from 377.0±80.0ms to 427.2±75.7ms ($\Delta = 50.2$, $p<0.01$) (Figure 20). This cycle length prolongation persisted in 3 of the 5 dogs following RAGP ablation, with an average cycle length increase of 31.7±25.7ms. There was no cycle length change with LAGP stimulation upon completion of Ao/SVC ablation.

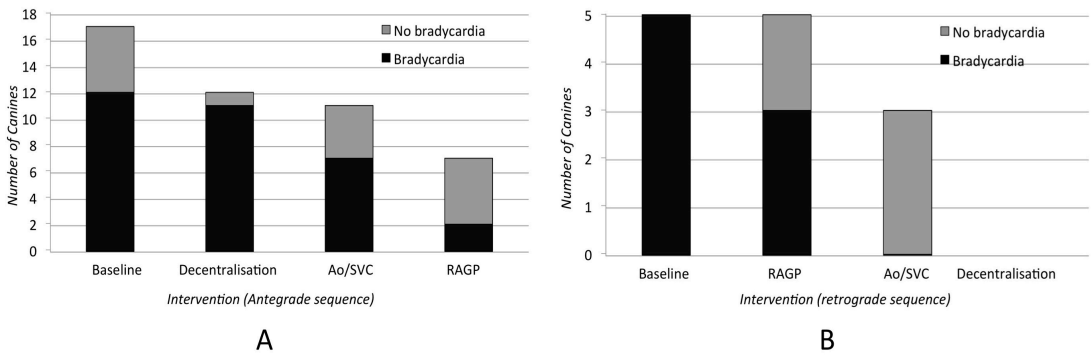

Figure 17. Number of dogs presenting bradycardic response to LAGP stimulation at baseline and following sequential ablations.

A - Antegrade group, B – Retrograde group. AO/SVC = periaortic and superior vena cava plexi. RAGP = Right atrial ganglionated plexus.

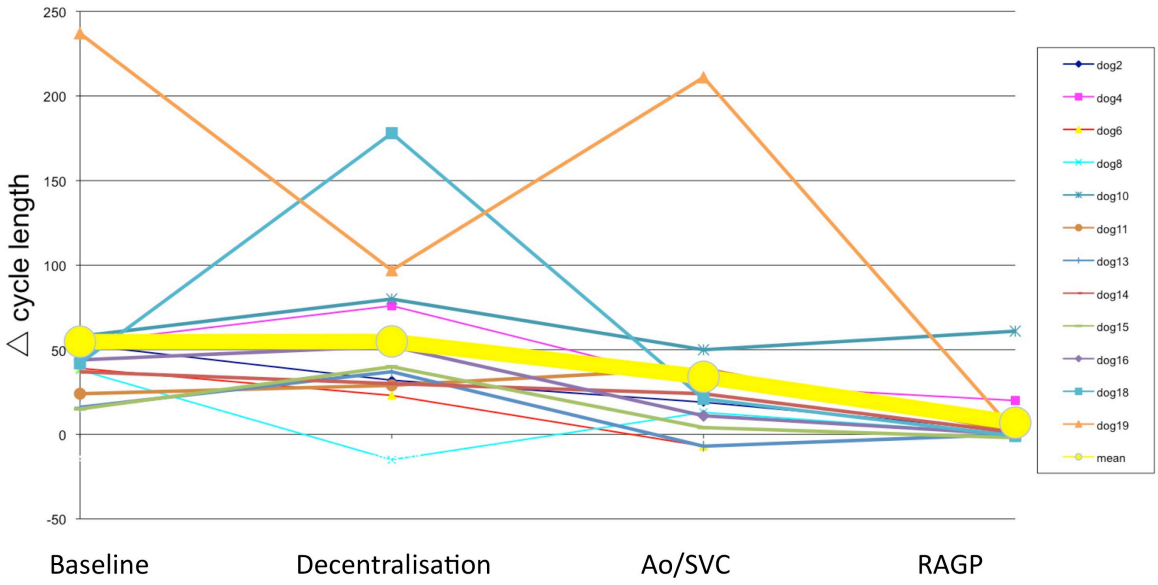

Figure 18. Average change in cycle length with LAGP stimulation following each step of the ablative protocol.

All animals demonstrating bradycardia at baseline are represented. The thick yellow line shows the mean cycle length change decrease throughout the experimental protocol.

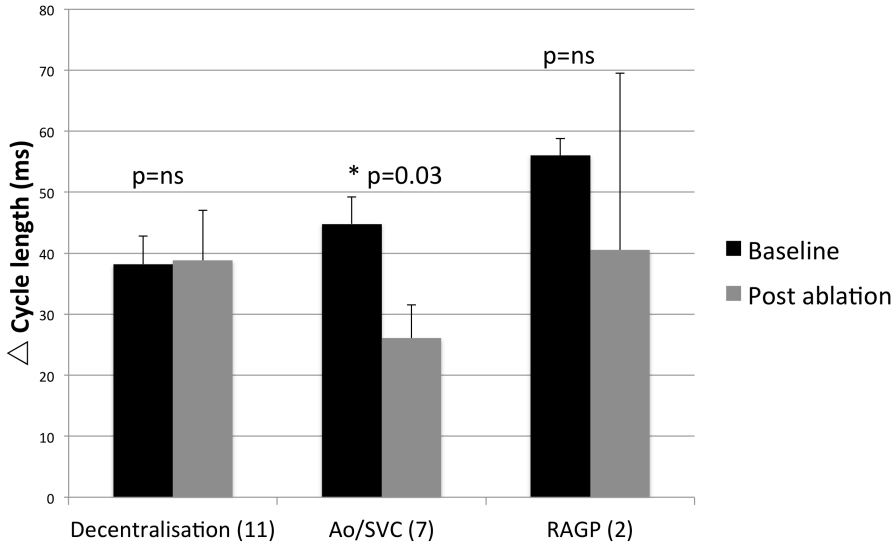

Figure 19. Average cycle length change per ablation sequence in the antegrade group.

Only preparations showing response at each level are included for analysis. A decline in cycle length change following periaortic/SVC ablation is noted.

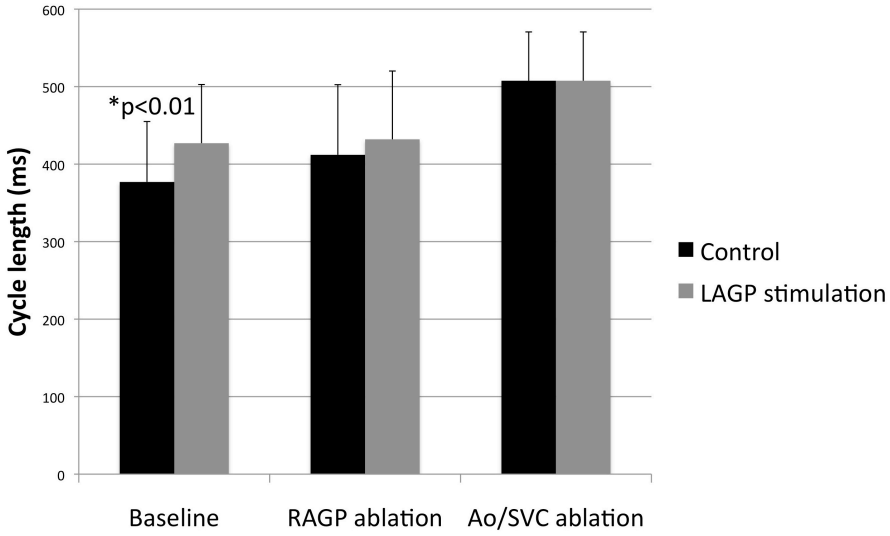

Figure 20. Average cycle length change in retrograde group, control versus LAGP stimulation

There was a significant prolongation of cycle length with LAGP stimulation at baseline. Following RAGP ablation, there was a trend toward prolonged cycle that did not reach statistical significance. Following Ao/SVC ablation, there was no change in cycle length.

3.2 **Isointegral distribution mapping**

3.2.1 Antegrade ablation sequence

Right atrial free wall

Isointegral mapping was available in the final 19 dogs (14/17 in the antegrade group, 5/5 in the retrograde group) because AV nodal blockade was not performed in the initial 3 preparations. Upon analysis, 14 of the 19 dogs with available repolarisation maps showed isointegral REPOLΔ in the right atrium, this includes 14 of the 15 dogs that had a bradycardic response to LAGP stimulation.

Figure 21 shows isointegral maps of two typical preparations. The upper series, d11, shows isointegral REPOLΔ that persist following decentralization and Ao/SVC ablation, however, the response was suppressed following RAGP ablation. The lower series, d10, clearly demonstrates that REPOLΔ in the right atrium persisted throughout the entire ablation protocol, including RAGP ablation. Similar to the decreasing bradycardic response, surface activation in the right atrium decreased throughout the antegrade ablation sequence. These changes can be seen in Figure 22. At baseline, REPOLΔ on the right atrial free wall (RAFW) covered 365±252mm^2. Following decentralisation and Ao/SVC ablation there was a decreasing trend to 266±283mm^2 and 285±328mm^2 respectively. Following RAGP ablation there was a statistically significant decrease to 53±106mm^2 ($p<0.03$). Cumulative incidence maps, Figure 23, depict the progressive decrease of REPOLΔ in the antegrade group throughout the protocol.

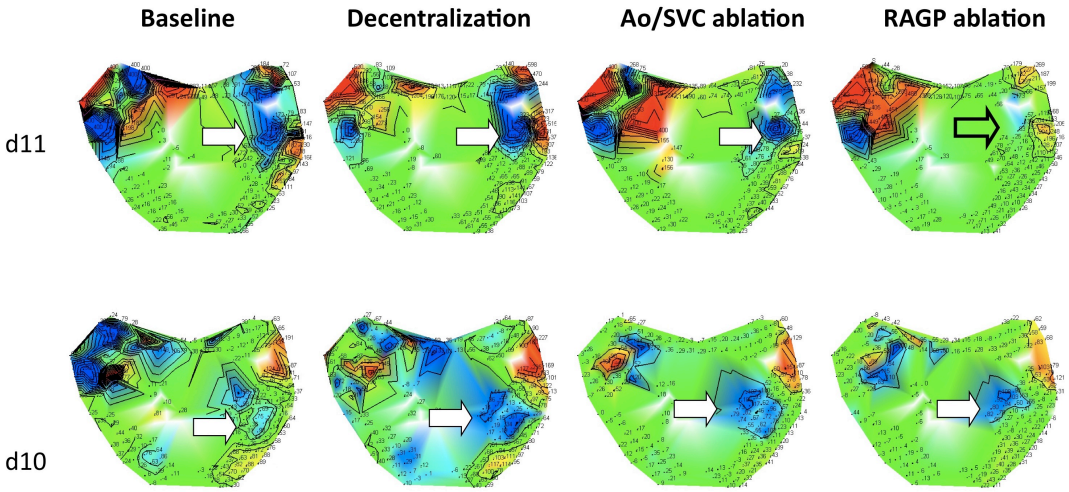

Figure 21. Examples of isointegral mapping with LAGP stimulation.

Isointegral changes in 2 representative preparations from the antegrade group are shown. At baseline, the positive isointegral changes in the right atrium (blue area) are consistent with the concomitant bradycardia. In the upper panel, REPOLΔ diminish progressively along the ablation sequence until disappearing after RAGP ablation. The lower panel shows a persistent response throughout all ablation, including the RAGP.

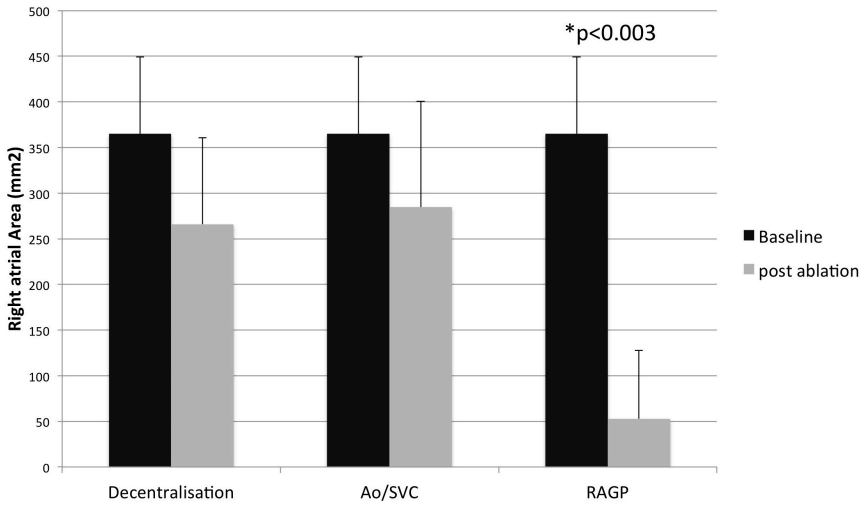

Figure 22. Surface area of activation along the right atrial free wall with progressive ablation in the antegrade group.

There is a trend of decreased surface area of activation following decentralisation and Ao/SVC ablation, while there is a statistical significance decrease following RAGP ablation.

Bachmann's bundle

There was a significant decrease in surface area of REPOLΔ over the entire atria following decentralisation compared to baseline (1706±747mm^2 vs. 1149±684mm^2, p<0.04). This change was most pronounced in Bachmann's bundle region. Figure 24 compares the surface area of REPOLΔ in Bachmann's bundle, comparing results for each ablative step to the previous one. Following decentralisation, REPOLΔ decreased from 295±213mm^2 to 178±171mm^2 (p<0.03). This area decreased further following Ao/SVC ablation to 93±97mm^2 (p<0.05).

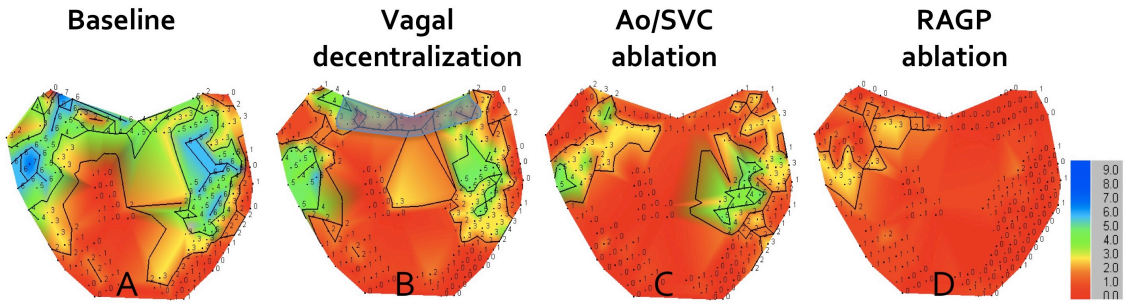

Figure 23. Cumulative incidence maps of REPOLΔ with antegrade group.

Repolarisation maps of 9 dogs represented. A progressive decline in surface area of atrial activation is observed, particularly in the right atrium. In map B, a significant suppression of REPOLΔ in Bachmann's bundle (shaded area) is noted following decentralization.

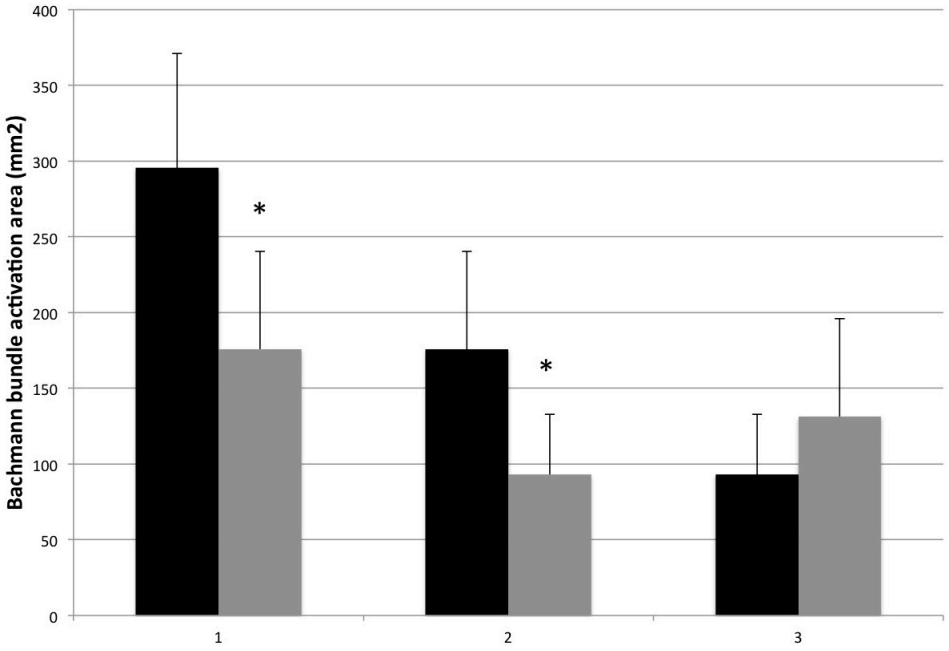

Figure 24. Isointegral changes in Bachmann's bundle throughout ablation sequence.

1 = significant decrease from Baseline (black) to decentralisation (grey). 2 = significant decrease from decentralisation to Ao/SVC ablation. 3 = insignificant change from Ao/SVC to RAGP ablation. * = $p<0.05$

3.2.2 Retrograde ablation

Surface area isointegral changes for the retrograde ablation group are presented in Figure 25. In addition to the bradycardic response, all dogs in the retrograde protocol demonstrated repolarisation in the right atrium as a response to LAGP stimulation at baseline (269±144mm^2). Following RAGP ablation, there remained important isointegral changes in the right atrium, although the surface area of those changes was significantly less than at baseline (269±144mm^2 vs. 124±158mm^2, $p<0.05$). REPOLΔ were almost completely abolished when the LAGP was stimulated following Ao/SVC ablation (4±9mm^2).

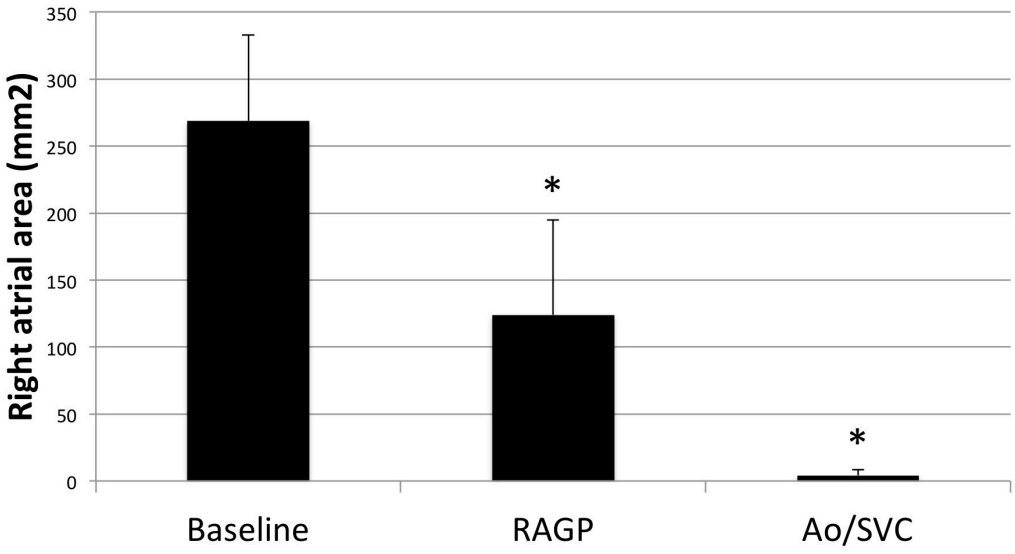

Figure 25. Surface area of activation along the right atrial free wall with progressive ablation in the retrograde group.

Following RAGP ablation, there was a significant decrease in average surface area of activation with LAGP stimulation. Isointegral changes were almost entirely abolished following Ao/SVC ablation, although this did not reach statistical significance when compared to changes post RAGP ablation. * = $p<0.05$ when compared to baseline.

Chapter 4. Discussion

The present study was designed to define the nature of the sinus bradycardia induced by LAGP stimulation in a canine model. The LAGP can be readily identified by the bradycardic response to stimulation in the majority of cases, with the RAGP being the primary, but not the exclusive, gateway to the sinus node. By progressively eliminating autonomic input into the heart, the pathways of this response were shown to be variable and overlapping. Stimulation of the LAGP has significant neural effects on the right atrium, while it is clear that they are not mediated primarily by a central reflex loop, rather by local pericardiac and intracardiac nerve fibres. Finally, neural effects in non-chronotropic regions, such as Bachmann's bundle, may be mediated by central reflex pathways between the LAGP and atrial tissues.

4.1 **Bradycardia**

A bradycardic response to LAGP stimulation was elicited in 17 of 22 dogs (77%), demonstrating that the LAGP can indeed be identified by the bradycardic response to electrical stimulation in the intraoperative setting. Butler et al first suggested the chronotropic properties of the LAGP in 1990 when they were able to elicit a bradycardic response in a significant number of canines with electrical stimulation.[168] Others authors have since duplicated these results but the phenomenon has not been studied systematically and the mechanism of this bradycardia has not been hypothesized previously.[150] Lu et al have gone the farthest in studying the pathway of the bradycardic response to LAGP stimulation, suggesting that the RAGP is the primary relay station to the sinus node, however bradycardic response to stimulation and AV nodal block were the only parameters studied.[169] The sequential ablation protocol in this study provides new information on the subject. Decentralisation by vagus nerve sectioning suppressed the bradycardic response in only one preparation and did not affect the degree of bradycardia in the remaining 11 dogs. Bulter et al had similar findings in a study of 8 canines, only two of which were affected by decentralisation.[168] This would

suggest that, with regard to the bradycardic properties of the LAGP, the vagus nerve, and as a consequence the central nervous system, play only a minor role. Thus it can be concluded that LAGP epicardial neurons are not uniquely afferent neurons with projections to the central nervous system, rather efferent neurons with pericardiac or intracardiac projections that can affect sinus node function. As will be discussed later, the central reflex arc may be implicated in nonchronotropic LAGP properties.

Following periaortic and SVC plexus ablation, the bradycardic response to stimulation was suppressed in 36% (4/11) of the remaining preparations in the antegrade group and 100% in the retrograde group (3/3) (Figure 17). In those preparations with a persistent bradycardic response, the average cycle change was decreased as compared to baseline. These findings are suggestive of two novel characteristics of the LAGP. 1) The periaortic/SVC plexi appear to be an important pathway in the transmission of neuronal signals from the LAGP to the sinus node, likely via the RAGP intermediary. 2) LAGP fibres likely travel along multiple pathways in a given heart. This is evidenced by the stepwise decrease in magnitude of bradycardia following periaortic/SVC ablation, suggesting truncated but not abolished LAGP input into the sinus node. While the periaortic/SVC plexi have been implicated as a significant autonomic neuronal pathway by several authors, this represents the first description of this pathway as it relates to the LAGP.[170-173]

It has been well documented that the RAGP is the chief input of the ANS and ICNS into the sinus node[117, 174-176]. More recently, several authors have suggested that the RAGP is the primary but likely not the only input sinus node.[115, 142, 177] The results of this study mirror those reports, clearly showing that the RAGP is not the only pathway by which the ICNS can influence the sinus rate. Furthermore, it has not been demonstrated previously that the LAGP has direct communications with the sinus node, independent of the RAGP. In the antegrade group, RAGP ablation suppressed the bradycardic response in five of the seven dogs that had maintained a vagal response following periAo/SVC ablation, while bradycardia persisted in two dogs even after RAGP ablation. The results of the retrograde group confirmed these findings, with three of five dogs maintaining a bradycardic response even after RAGP ablation. Figure 26 summarizes the multiple LAGP pathways that are

responsible for the bradycardic response to stimulation. This new information lends itself to the idea that the LAGP contains primarily afferent neurons with local pathways that can affect cardiac performance on a beat-to-beat basis without passing through the central nervous system.

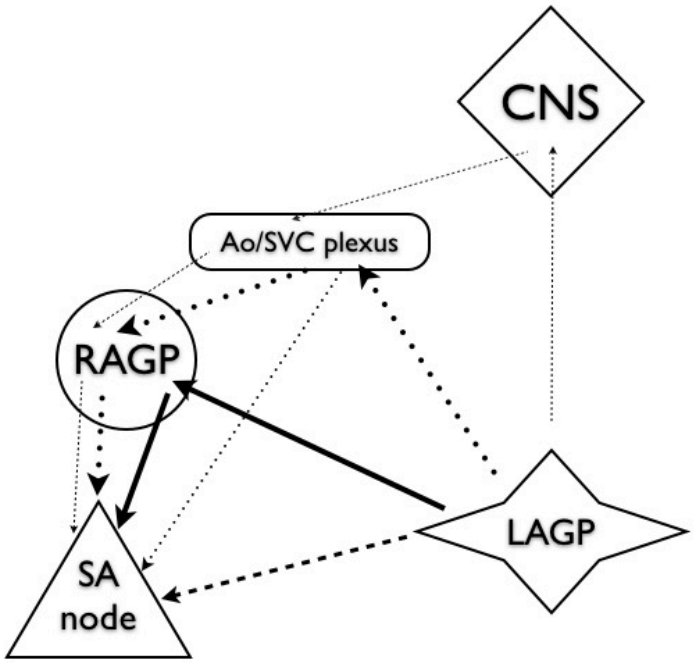

Figure 26. Summary of LAGP pathways responsible for sinus bradycardia.

Autonomic bodies include the central nervous system (CNS), periaortic and superior vena cava plexus (Ao/SVC plexus), right atrial ganglionated plexus (RAGP), and the sinoatrial node (SA node). Solid and dotted lines represent primary and secondary pathways, respectively.

4.2 **Right atrial repolarisation changes**

In classical studies documenting the effects of ganglionated plexus stimulation, only chronotropic and dromotropic parameters were used as markers of neural effects. Such limited endpoints risk missing effects of neural projections remote from the SA and AV nodes. By instrumentation with epicardial mapping electrodes and measurement of REPOLΔ, Pagé et al have been able to identify neural effects in many more atrial sites than identified previously.[141, 142]. It has been shown that positive integral deflections correspond to significant changes in action potential duration and allows us to identify areas affected by nerve stimulation.[178]

This study is the first to focus on the LAGP while evaluating isointegral REPOLΔ in addition to the classical chronotropic response. The results clearly demonstrate diffuse REPOLΔ across both atria (Figure 23), although not of the same magnitude as previously reported with vagal stimulation.[141] Importantly, there are significant REPOLΔ seen in the right atrium, corresponding to the concomitant bradycardia discussed above. In the antegrade group there was a trend toward decreasing surface area of REPOLΔ throughout the ablative protocol, with a significant decrease following RAGP ablation (Figure 22). In the retrograde group, surface area of REPOLΔ decreased significantly following RAGP and Ao/SVC plexus ablation (Figure 25). The implications of these finding are two-fold. 1) This confirms that the bradycardic response witnessed with LAGP stimulation is indeed the result of neural changes in atrial tissue, providing the physioanatomic basis for the "vagal" response when electrical stimulation is carried out both experimentally and clinically.[150, 152, 155, 179, 180] 2) As with the bradycardic response, the stepwise decrease in surface area of REPOLΔ reveals the complex nature of LAGP-atrial interactions. Although the RAGP is an important mediator of LAGP effects on the right atrium, there are other pathways, both pericardiac and intracardiac, through which the LAGP can modulate cardiac physiology.

In one preparation there was no bradycardic response recorded with LAGP stimulation, however, there were significant repolarisation changes seen upon analysis of epicardial electrode maps. The neurophysiologic basis of this anomaly can only be hypothesized at this point. It is possible that this phenomenon was present in each of the five preparations that did not produce a bradycardic response but because of the experimental design we were unable to isolate the repolarisation changes. The atrial electrograms are recorded in real time during the experimental protocol, however, due to time constraints while working with a live animal, the isointegral maps are not analysed until a later time. As a consequence, in the absence of bradycardia the choice of electrograms for analysis is somewhat arbitrary and can possibly miss an LAGP activation that may have occurred during an earlier or later stimulation. This example brings to light our limited comprehension of the complex functions of the ICNS. Each ganglionated plexus may serve a multitude of purposes

related to chronotropy, dromotropy, inotropy, or any number of other cardiac neurophysiologic regulatory roles. It is important to keep this in mind when considering targeted ablation of the ICNS in a clinical therapeutic setting. This concept will be elaborated upon in a subsequent section.

4.3 **Bachmann's bundle**

Since it was first identified in 1916, Bachmann's bundle has become known as the primary interatrial conduction pathway.[77, 81, 181-183] More recently, it has been experimentally linked to the initiation and propagation of atrial tachyarrhythmias.[140, 182] In the present study, LAGP stimulation caused significant neurally mediated repolarization changes in Bachmann's bundle, as well as in other atrial regions. Interestingly, while the changes in Bachmann's bundle were significantly diminished in the antegrade group following decentralization, there was no difference in the bradycardic effect of LAGP stimulation. This suggests that although primarily local pathways, as detailed above, mediate the bradycardic response to LAGP stimulation, the neurally induced repolarization changes in Bachmann's bundle are largely mediated by a central reflex arc via the vagus nerves. Previous studies detailing atrial repolarization changes in Bachmann's bundle have found significant neurally induced changes associated with stimulation of a variety of mediastinal nerves.[140, 141] Pagé et al found repolarization changes in the area of Bachmann's bundle with stimulation of the right vagosympathetic complex and the superior vena cava.[141] These changes were associated with early bradycardic beats preceding the onset of atrial tachyarrhythmia. These findings expose the significant neural input from central nerves into Bachmann's bundle. Our results support the notion of considerable central innervation of this area, however, they also once again allude to our limited understanding of the intrinsic cardiac nervous system as a whole. While LAGP stimulation resulted in significant neural repolarization changes that were modified by vagus nerve sectioning, we do not know how these changes affect the autonomic nervous system or the heart on which they occur. The innervation of Bachmann's bundle highlights the impressive interconnectivity and redundancy of the ICNS. With this in mind, it is difficult to imagine how selective

neuroablation, through localized ganglionated plexus ablation, can have a clinically relevant effect on arrhythmia formation of preservation.

4.4 **Intrinsic cardiac nervous system remodelling**

The experiments described in this thesis were performed on healthy canines with normal atrial tissue. In the presence of disease, such as ischemia, heart failure or arrhythmia, the ICNS can undergo significant remodelling, either physiological or anatomical, that may alter its response to otherwise benign physiologic stimuli. This phenomenon is well described by Armour and is supported by several experimental studies.[105, 184-188] It has also been demonstrated that therapeutic interventions, such as spinal cord stimulation and transmyocardial laser revascularization, can have lasting effects on the ICNS.[187, 188] The current study adds to our knowledge of the neuroanatomy of the ICNS, providing another element to our global understanding of this complex system. Further work is needed in order to integrate this information into a model of pathologic atrial remodelling. Remodelling can contribute to or exacerbate cardiac disease by several mechanisms. and thus react differently to stimuli. out a history of arrhythmia and therefore a atrial fibrillation remodelling

4.5 **LAGP ablation in treatment of atrial fibrillation**

The results of the current study uncover important information regarding the functional anatomy of the LAGP. Although this work cannot be extrapolated to a conclusion regarding the arrhythmogenic potential of this group of neurons, understanding the basic workings of the LAGP is particularly important in light of the increasing role it plays in the interventional treatment of AF.

Several authors have explored the arrhythmogenic potential of the LAGP both experimentally and clinically. Despite the growing number of clinical reports describing LAGP ablation in the treatment of AF, there is surprisingly little experimental evidence supporting this practice. Lu et al investigated the effects of LAGP ablation on AF threshold and found that the threshold for AF induction was higher following ablation of a variety of ganglionated plexi, including the LAGP.[189]

The methodology of this study may be challenged because a bipolar ablation probe was used to carry out the ablation, which essentially studies the effect of transmural ablation lines in the area of the pulmonary veins rather than isolated ablation of ganglionated plexi. Although this study may not prove the arrhythmogenic potential of the LAGP, it is in line with the rest of the AF literature advocating pulmonary vein ablation for the treatment of AF. Lemola et al recently published more convincing results with regard to the role of the LAGP in AF formation.[190] While studying thirty mongrel dogs, they showed that intact pulmonary vein-left atrial connections were not necessary to maintain vagal AF, yet epicardial ablation of ganglionated plexi suppressed vagal responses. Despite their findings, the authors do caution that selective ablation alone is likely not as effective as a combined approach with pulmonary vein circumferential ablation and vagal denervation.

Clinical studies have reported generally favourable results with ganglionated plexus ablation but also give reason to proceed with caution. Pokushalov et al reported two-year follow-up of ganglionated plexus ablation with a success rate of 38% in patients with persistent AF and slightly better results when combined with pulmonary vein isolation.[191] These results suggest some effect of ganglionated plexus ablation but are certainly disappointing as a therapeutic intervention. The same group reported better results in patients with paroxysmal AF, with 71% in sinus rhythm at twelve months. Interestingly, they did not attempt to identify ganglia through the bradycardic response but instead employed an "anatomical" ablation approach, applying a radiofrequency probe to the sites where autonomic ganglia have been reportedly been located in the literature. As the authors state, the more common practice of high frequency stimulation to guide ablation may illicit responses in local autonomic nerves rather than the autonomic ganglia themselves and therefore may not be a suitable marker on which to base ablative interventions.

Other authors have reported pulmonary vein isolation with a minimally invasive approach with the addition of ganglionated plexus localisation by high frequency stimulation and subsequent ablation. Success rates vary from 77-87% for paroxysmal AF and are significantly lower for persistent AF.[179, 192, 193] A difficulty with each of these studies is that there is no evidence that selective ganglionated

plexus ablation had any bearing on the freedom from AF, in other words, pulmonary vein isolation alone may have yielded the same outcome. In fact, there is some evidence that selective denervation following identification with high frequency stimulation may be deleterious by causing heterogeneity in the atrial tissue, an idea that has been echoed by several authors.[180, 194, 195] The effect of the complete Maze procedure on the ICNS is difficult to qualify and has not been systematically studied. The extensive nature of ablation lines likely effect some degree of ICNS remodelling, but further work must be done to better understand these interactions. There have been a limited number of clinical studies suggesting that patients can benefit from the addition of ganglionated plexus ablation to the conventional Maze procedure.[196] Although this data is far from conclusive, it would suggest that the Maze procedure alone does not adequately address arrhythmias originating in the ICNS.

The present study demonstrates that the LAGP likely does contain efferent neurons that modulate cardiac neurophysiology locally and therefore could theoretically be involved in arrhythmia formation. However, the complex pathways described above open a small window into the previously demonstrated complexity of the ICNS. This should raise a concern that selective neuroablative interventions may not produce the desired effect if based on an oversimplification of this elaborate and somewhat unpredictable network of neurons.[112, 177] Several avenues of study must be completed before determining the role that these ganglionated plexi play in arrhythmia formation. These must include chronic animal models permitting the evaluation of atrial remodelling with and without ganglionated plexus ablation. The results of an ongoing study in our laboratory may shed some light on this subject. In a chronic dog model with rapid atrial pacing, the effect of bilateral anatomical ganglionated plexus ablation on atrial refractoriness and AF induction is being evaluated. The results, whether positive or negative, should enhance our knowledge regarding this important topic.

4.6 **Limitations**

When using animal models to simulate human anatomy and pathology, one should always be cognizant of important interspecies differences. Regarding the ICNS, anatomic studies have demonstrated adequate correlation between canines and humans, and pathophysiologic reactions to various stimuli tend to correspond adequately.[96, 114] One concern in this particular model was time as a confounding variable in the decreasing bradycardic response. Two sham procedures were performed to assess whether the decreasing bradycardic response was simply due to time related fatigue of the neuronal response. In these procedures, the bradycardic response to stimulation persisted throughout, implying that the diminishing or extinguished bradycardic response in the study group was in fact a result of the sequential ablation sequences. Additionally, no histologic or pathologic analysis of atrial tissue was completed to verify the presence of neurons in the stimulated areas. Although this may have been an interesting adjunct to the experiments, it was unnecessary because stimulation was performed during the absolute atrial refractory period, thus ensuring neuronal and not myocardial activation. Transmurality of radiofrequency ablation was not verified, however, this was not essential since ganglionated plexi are known to lie primarily on the epicardial surface of the heart. Finally, while the LAGP is likely present in all canines, we were unable to demonstrate neurally mediated repolarization changes in 33%. Even though it is possible that repolarization changes occur in the absence of chronotropic changes, we were dependent on the bradycardic response to stimulation as a sign of LAGP activation. While this is an inherent limitation of the model, it is representative of what can occur clinically when intraoperative ganglionated plexus stimulation is performed. Despite these pertinent limitations, the results described above remain applicable and persuasive.

Chapter 5. Conclusion

This study attempted to define the nature of the sinus bradycardia induced by LAGP stimulation in a canine model. We have shown that LAGP stimulation has significant effects on the right atrium. By progressively eliminating autonomic input into the heart we were able to show that the pathways of this response are variable and overlapping. It is clear that the bradycardic response is not mediated primarily by a central reflex loop, rather by local pericardiac and intracardiac nerve fibres. The periaortic/SVC plexi are important in relaying neuronal signals from the LAGP to right sided structures, and there may even be a direct line of communication between the LAGP and the sinus node. In addition to confirming the marked neuronal effects of the LAGP on the right atrium, mapping atrial repolarization changes highlighted the complexity of the neuronal projections by unmasking the suppression of repolarization changes in Bachmann's bundle following decentralization. These findings suggest the presence of both afferent and efferent neurons in the LAGP. As this pertains to the current trend in the treatment of AF, notably the adjunctive targeted ganglionated plexus ablation procedures, our findings maintain the possibility that ablation of these neurons could affect arrhythmia inducibility. However, although we have been able to elucidate the pathways of these nerve fibres, their function and role in atrial fibrillation remains to be determined. Even if these plexi prove to have arrhythmogenic potential, the ideal ablation technique is yet to be determined. Owing to the overlapping and complex nature of the cardiac autonomic nervous system, focal ablation within ganglionated plexi may not produce the desired effect of arrhythmia termination. Rather, a more global approach to cardiac denervation may be indicated.

Chapter 6. Bibliography

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