The autonomic nervous system and cardiac arrhythmias: current concepts and emerging therapies

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Abstract | Research into cardiac autonomic control has received an explosion of interest in the past 20 years, and we are now at a critical juncture with regard to the clinical translation of the experimental findings. There has been a rush to develop clinical interventions and implant a range of devices aimed at cardiac neuromodulation therapy. This interest has been driven by research, superimposed on commercial opportunities and perhaps the more relaxed regulatory framework governing implantable devices and interventions compared with that for pharmacotherapy. However, many of the results of the clinical trials into these therapies have been disappointing or conflicting. This lack of positive results is partly due to a scramble to find simple solutions for complex problems that we do not yet fully understand. Are there reasons to be optimistic? In this Review, we highlight areas in the field of cardiac autonomic control that we feel show most promise for clinical translation, and areas where our current range of blunt tools need to be refined to bring about long-term success in treating arrhythmia.

[H1] Introduction

Neurologists have known for many years that brain injury can cause cardiovascular complications[1]. These complications can range from disturbances in blood pressure to marked alterations in the electrocardiogram leading to cardiac arrhythmias. But even without neurological trauma, the autonomic nervous system (ANS) is a powerful modulator of cardiac excitability when the body faces physiological, psychological and pathophysiological stress[2], given the neural connection between the brain and the heart, and the heart's own intrinsic nervous system — the 'little brain in the heart' [3]. Indeed, the origins of emotions controlling the heart and the heart sensing emotion are embedded in folklore and literature; 'frightened to death', 'died of a broken heart', 'matters of the heart', 'heart-felt'. Is this 'neuro-mythology' or is there a neurophysiological basis to these sayings that presents a therapeutic opportunity to target the cardiac–neural axis?

During the past 45 years, our improved understanding of the neuroanatomical circuits at the level of the heart has resulted in the re-emergence of the idea that guided therapy to the ANS can be effective in treating arrhythmia. These therapeutic strategies have involved either neurostimulation, surgical resection or ablation of ganglia, or gene therapy that is targeted to cardiac neuro-effector sites [4,5,6,7,8,9] (Figure 1). This Review discusses the neuroanatomical and neurophysiological basis for neuromodulation therapy, and also assesses the latest clinical trial data on the clinical efficacy of this approach.

[H1] Integrated cardiac neuroanatomy

The ANS is responsible for fine tuning the activity of the heart to meet the needs of the entire circulatory system and organism, as its behaviour varies during periods of rest, stress and exercise[10]. The classical view of the sympathetic and parasympathetic branches of the ANS functioning as the 'accelerator and brake' are over simplistic given what we now know about the complex neuroanatomy and physiology of the ANS[11]. A series of elegant experiments, pioneered by investigators such as Ardell and Armour[3], have revealed an interconnected cardio–neural hierarchy. As shown in Figure 2, level 1 in this hierarchy includes higher cortical centres as well as the brainstem and the spinal cord. Level 2 includes all intrathoracic extracardiac neurons (including the stellate ganglia **[G]**)[12,13], and level 3 encompasses all the intrinsic cardiac neurons[3]. Afferents within the heart and circulation provide sensory signals to levels 1 and 2 for processing[14,15]. This way, the excitability of efferent cardiac parasympathetic or sympathetic pathways therefore depends on tonic inputs at several levels in the brain, spinal cord and in the extrinsic and intrinsic cardiac ganglia[16], as illustrated in Figure 2.

In the setting of some forms of hypertension, acute myocardial infarction (MI) and chronic heart failure (HF), neurophysiological changes occur at all levels of the neural hierarchy[17,18]. At the level of the heart, scar formation and fibrosis[19], particularly in the border zone of infarcts[20], contribute to conduction block[21] and slowly conducting pathways lengthened by branching and merging bundles of surviving cardiomyocytes[22]. These alterations create a fixed substrate for re-entrant arrhythmia. In addition, neural remodelling and heterogeneity of innervation can also superimpose a dynamic arrhythmogenic substrate[23-25]. Afferent-mediated activation of neurohumoral systems also increases sympathetic drive and reduces vagal tone[26], which in the short term can facilitate the maintenance of cardiac output[27]. However, this maintenance of cardiac output is at the expense of increased myocardial oxygen demand and Ca^{2+} overload in cardiomyocytes, leading to ectopic activity and structural remodelling, causing hypertrophy. Chronic, abnormal cardiac afferent signalling reinforces the maladaptive response of persistent sympathetic activity[28] and loss of cardiovagal protection[29], leading to the progression of cardiac disease, including increased likelihood of pump failure and arrhythmia-induced sudden death.

Although these sweeping statements are broadly true, the complexity within the 'heart's little brain', and indeed at higher levels of the neural hierarchy, should not be underestimated. The neuronal connections are not as simple as highlighted in diagrams such as the one shown in Figure 2, although these signalling loops undoubtedly exist. The neural circuits underpinning these pathways are still poorly represented in 3D reconstructions and the electrophysiological characterization in normal and diseased states is incomplete (Figures 3 and 4). Immunohistochemistry shows that neurons within the intrinsic cardiac plexus are mostly cholinergic, phenotypically complex, and stimulation at discrete sites identifies clusters capable of independent and selective effects on cardiac electrophysiology[30]. Neuronal recordings and immunostaining demonstrate the presence within the intrinsic cardiac plexus of local circuit neurons and afferents that respond to different mechanical and chemical stimuli, as well as parasympathetic[31,32] and a few sympathetic[33] efferents.

The principal cells in the intracardiac ganglia send axonal projections to pacemaker cells, the cardiac electrical conduction system and the contracting myocardium. Within the ganglia, some cells are interneurons that terminate in the ganglion itself, while other axons innervate other ganglia within the plexus. Sympathetic postganglionic efferents pass through and can also terminate within the intracardiac ganglia. Moreover, the ganglia receive a rich afferent innervation, and together with small intensively fluorescent cells that interact with interneurons and principal cells, collectively add to the network complexity[34]. Furthermore, the network can remodel both structurally[35] and behaviourally[36] in disease states (Figure 5a). For example, epicardial application of resiniferatoxin to activate and destroy cardiac afferents that express vanilloid receptor 1 (VR1) enhances sympathetic outflow in normal pigs[37]. Yet in a rodent model of chronic HF, the same intervention reduces cardiac and renal sympathetic nerve activity and improves

cardiac remodelling[29,38]. Moreover, the physiology of different neurotransmitters and co-transmitters cross communicating between different neuronal populations further complicates data interpretation. Following myocardial injury, the process of reinnervation and the release kinetics of classical neurotransmitters and co-transmitters, superimposed on neurotransmitter switching, are only just beginning to be appreciated[16,39,40].

[H1] The ANS and ventricular arrhythmias

Given the millions of normal heart beats that occur over a lifetime, it seems surprising that even in patients with cardiac pathology only on very rare occasions are abnormal rhythms generated. Even then, these abnormal rhythms can be brief and asymptomatic whereas on other occasions, they can be sustained and life-threatening. A useful framework for understanding arrhythmias is that they initially require a trigger to generate an extra-stimulus. This extra-stimulus needs to have the right properties and be perfectly timed within a vulnerable window to generate an ectopic beat. Then, a suitable substrate needs to be present within the heart to maintain the propagation of the ectopic beat. The substrate can be structural and/or electrophysiological and static and/or dynamic. Ventricular tachycardia (VT) is formed from a single re-entrant circuit, but if fragmentation or wave-break occurs, then the single spiral can form multiple wavelets and fibrillation[41,42].

Each step in the process of arrhythmogenesis is probabilistic, with all criteria only rarely being fulfilled, although the ANS can influence every stage[43]. Overall, both acute MI and chronic HF are associated with sympathetic hyperactivity and vagal withdrawal in experimental animal models[44-47] and large-scale human studies. In human studies, plasma catecholamine levels, heart rate variability and baroreflex sensitivity are predictive of morbidity and mortality[48,49,50]. In these conditions, ventricular arrhythmias tend to occur during exercise, first thing in the morning or during REM sleep when sympathetic drive is highest[51,52]. Baroreflex sensitivity[53] and heart rate recovery following exercise[54] are also important modifiers of the risk of arrhythmia in patients with long QT syndrome (LQTS) type 1 (which is due to mutations in *KNCQ1*, encoding the channel responsible for the slow component of the delayed rectifier K^+ current, I_{Ks}). Thus, understanding spatio-temporal features of changes in cardiac innervation continue to be the subject of study as investigators probe neural targets as an alternative therapeutic approach in modifying the risk of ventricular arrhythmia.

[H2] Arrhythmic triggers and β-adrenergic receptor stimulation. Arrhythmic triggers include afterdepolarisation and abnormal automaticity. The latter can be driven by Ca^{2+} release from the sarcoplasmic reticulum, which drives membrane depolarization, or by local currents from ischaemic regions that depolarize

neighbouring zones. Most pathological arrhythmias are triggered by a delayed afterdepolarization (DAD), which results from an overloaded Ca^{2+} store in the sarcoplasmic reticulum that causes spontaneous diastolic Ca^{2+} release driving electrogenic Na–Ca^{2+} exchange (NCX) current, which then depolarizes the membrane towards the action potential threshold[55,56]. This Ca2+ overload is exacerbated by β-adrenergic receptor stimulation of adenylyl cyclase[57] leading to elevations in cAMP levels[58], which in turn increases intracellular Ca^{2+} loading via protein kinase A (PKA)-mediated increase in the L-type Ca^{2+} current (I_{CaL})[59] and removal of phospholamban-mediated inhibition of the sarcoplasmic reticulum Ca^{2+} ATPase 2 (SERCA2)[60]. In the border zone of a MI scar, remodelling of ion channels, transporters and Ca^{2+} calmodulin dependent kinase II (CaMKII) signalling influences Ca^{2+} handling[61] and can predispose to an arrhythmic trigger[62].

Early afterdepolarizations (EADs) are more common when the action potential is prolonged, as occurs in inherited LQTS, hypokalaemia and hypocalcaemia, or in the presence of drugs that block K^+ channels. EADs are more common during extremes of bradycardia, when the action potential is longer or during episodes of tachycardia if the action potential duration (APD) is not shortened, and are, therefore, influenced by autonomic control of heart rate and APD[63]. The mechanism for EADs during the plateau phase of the ventricular action potential is classically thought to be due to reactivation of the I_{CaL}[55,64]. Like DADs, EADs are also more common during conditions of intracellular Ca^{2+} overload, because the I_{CaL} can be enhanced by high intracellular Ca^{2+} levels and by L-type Ca^{2+} channel phosphorylation by CaMKII[65] and can, therefore, also be influenced by the ANS independently of the heart rate.

[H2] Re-entry and adrenergic modulation of refractoriness and restitution. Atrial or ventricular ectopic beats are common in otherwise normal healthy hearts, in which the ectopic beats rarely lead to arrhythmias. The ectopic beats have to be perfectly timed during a vulnerable window and need to have a suitable substrate such that the wave of excitation they produce can form a self-sustaining circuit (circus or spiral wave). Cardiomyocytes emerging from their refractory period **[G]** are then re-excited prematurely by the return of the local excitation wave (re-entry**)**. A slow conduction velocity of wave propagation favours re-entry, as does a short refectory period, because it allows time for the cardiomyocytes to regain excitability. β-Adrenergic receptor stimulation is well known to shorten the refractory period by increasing the I_{Ks} and shortening the APD[66], and the effects of stellectomy and vagotomy on ventricular refractoriness were first described *in vivo* in dog models[67].

The shorter the APD and refractory period of an ectopic beat, the more likely it is to be able to produce a re-entrant circuit. The relationship between APD and the preceding diastolic interval is known as the electrical restitution properties[68]. The effect of β-adrenergic receptor stimulation on electrical restitution has been studied across the ventricular epicardium both *in vitro*[24,25] and *in vivo*[69], and transmurally in ventricular

wedge preparations[70] thanks to multielectrode arrays and optical mapping techniques. Sympathetic stimulation produces regional apex–base changes in restitution kinetics[24,25,71], which are not seen in response to infusions of exogenous noradrenaline[72], and arise from differences in regional innervation. This heterogeneity in recovery of excitability has the potential to be pro-arrhythmic. Focal epicardial application of noradrenaline causes triggered activity and computational modelling suggests that adrenergic-induced Ca^{2+} overload can activate the NCX current. This activation can drive more positive charge, pushing the membrane potential closer to the threshold for depolarization[73]. This idea that heterogeneity or gradients of activation are pro-arrhythmic is further supported by studies using optical mapping techniques in innervated, isolated, rabbit hearts[74].

[H2] A sustaining substrate for re-entry and the sympathetic nervous system. Re-entry requires a central area of conduction block, which can be anatomical or functional, and also unidirectional conduction block or refractoriness at initiation, such that both waves of excitation around the area of central conduction block do not collide and extinguish each other. Re-entry, therefore, requires an appropriate substrate[75]. A substrate can be acquired during local ischaemia, which uncouples gap junctions and reduces the resting potential thereby preventing some fast Na^+ channels from recovering from the inactivated state, leading to slowed conduction[76]. Overtime, the substrate can become anatomically fixed, with thin, slow-conducting fibres of cardiomyocytes within the infarct scar that can promote re-entry[77]. This risk of re-entry is exacerbated by changes in the expression of membrane ion channels and gap junction proteins in the infarct border zone[78], which can slow conduction. Structural abnormalities which may act as a substrate can also arise due to genetic conditions such as in arrhythmogenic right ventricular cardiomyopathy, hypertrophic cardiomyopathy and inherited dilated cardiomyopathy (particularly lamin A/C mutations)[79]. Substrates also have a dynamic component because electrophysiological properties can be influenced by hypoxia, haemodynamic, metabolic and ionic changes, drugs and autonomic tone. For example, whereas stimulation of stellate ganglia increases conduction velocity[80], modulation of propagation after MI is heterogeneous in the healed scar, with some regions having paradoxical conduction slowing, promoting an arrhythmogenic substrate in the peri-infarct region[23].

The sympathetic innervation of an infarct border zone also undergoes substantial remodelling, with denervation occurring immediately after infarction, followed by subsequent reinnervation[81]. The exact contribution of sympathetic reinnervation in the genesis of arrhythmia in the infarct border zone is yet to be fully determined. Preventing denervation of the infarct border zone through protease inhibition does not seem to influence the risk of arrhythmia[82], whereas preventing denervation by targeting neuronal protein tyrosine phosphatase receptor-σ (PTPσ) seems to reduce the risk of arrhythmia[83]. Chondroitin sulfate proteoglycans present in the infarct scar tissue prevent sympathetic reinnervation by binding to PTPσ, but

absence or modulation of PTPσ restores innervation[83]. However, knocking out the gene encoding PTPσ can also be pro-angiogenic[84] and improve revascularization. Interestingly, promoting reinnervation of the infract border zone through nerve growth factor (NGF) infusion in the stellate ganglia after MI results in an increased incidence of ventricular arrhythmia and sudden cardiac death[85]. In a dog model, NGF levels increased after MI, leading to heterogeneous hyperinnervation[86]. However, the interpretation of this observation might be more complex because NGF can influence the development of afferent neurons whose activity is stimulated by acute ischaemia[87]. Evidence suggests that the neuropathy associated with diabetes mellitus can also extend to cardiac afferent innervation and contribute to, and this arrhythmogenic neuropathy can be reversed by *Ngf* gene transfer[89]. The role of different neurotrophic factors in setting the balance between improved afferent sensing and worsening heterogeneous efferent innervation after MI deserves further study because it might reveal novel therapeutic opportunities.

Comparing these animal studies with clinical imaging studies using catecholamine analogues such as meta-iodobenzylguanidine (ADMIRE-HF trial[90]) and 11C-metahydroxyephedrine (PARAPET trial[91]) is interesting. The findings of these studies suggest that both the total volume of denervated myocardium and the volume of viable denervated myocardium after infarction predict ventricular arrhythmias and sudden cardiac death. These observations might be explained if excessive hyperinnervation or denervation produced similar electrophysiological effects and were equally pro-arrhythmic[92]. Regions of hyperinnervation were observed in explanted hearts from patients with a history of ventricular arrhythmia who received a heart transplant[93]; however, these regions were not the predominant phenotype in the border zone of the injured myocardium, but rather local findings at certain border zones, suggesting that a combination of denervation and hyperinnervation occurs. Alternatively, the border zone of the injured myocardium might be structurally hyperinnervated in terms of nerve density, but functionally behave as if denervated. Interpreting studies is further complicated by the observation that both β-adrenergic receptor super-sensitivity [83] and downregulation[94] have been observed in the border zone of the injured myocardium. In ventricular wedge preparations from failing human hearts, β_2-adrenergic receptor stimulation seems to have a more prominent role when β_1-adrenergic receptors are downregulated, shortening the APD and increasing transmural heterogeneity[70].

As a MI scar heals and chronic HF develops, changes in other inflammatory and neurohumoral pathways, including a range of growth factors and cytokines, can also become involved. These factors include members of the IL-6 family and leukaemia inhibitory factor[10], which via gp130 signalling pathways can induce a phenotype switch in neurons from an adrenergic to a cholinergic phenotype. Around 2 weeks after MI, acetylcholine levels in the infarct border zone transiently increase as sympathetic neurons start to express and release acetylcholine and noradrenaline[95,96]. In chronic HF, histological and molecular analysis of stellate ganglia from animal models[97] and humans[98] demonstrate increased staining for the synthetic enzymes of

norepinephrine such as tyrosine hydroxylase as well as evidence of inflammation, neurochemical remodelling, oxidative stress, and satellite glial cell activation[99]. In a chronic MI model, increased expression of neuronal nitric oxide synthase (nNOS) has been observed in the ventral interventricular ganglionated plexus, dorsal root ganglia and stellate ganglia[35]. These changes might be a hallmark of early compensation involving nitric oxide (NO) pathways to modulate sympatho-vagal balance[100].

[H2] Parasympathetic influence on ventricular arrhythmias. Although cholinergic innervation of the ventricles is relatively sparse compared with the ganglionic plexi in and around the atria, epicardial and endocardial ventricular innervation has been observed across a range of mammalian species[101-104]. Stimulation of the left cervical vagus nerve can decrease left ventricular contractility in pigs and humans[105], although the sites of preganglionic nerve terminations in parasympathetic ganglia varies across species[104,106,107]. Of note, the cervical vagus nerve is composed of up to 70% afferent fibres[108], which can also be recruited during stimulation. For example, if the vagus nerve is not transected, vagus nerve stimulation (VNS) can activate afferent fibres, with accompanying reflex inhibition of cardiac electrophysiological and haemodynamic effects[109] The experimental approach, as well as stimulation parameters and patterns used, are therefore critical in determining the overall physiological response[110].

Much has been written about the experimental basis and clinical implications of the role of the cardiac vagus nerve in the control of heart function[111,112]. The protective effect of the vagus nerve on the heart has been recognised since 1859[113], and many studies have demonstrated that activation of the vagus nerve can prevent ventricular arrhythmia both in the normal heart and in the context of acute ischaemia and reperfusion[114-118]. Some of the most convincing evidence has come from studies by Peter Schwartz and colleagues. These researchers showed that in dogs with a healed MI, occlusion of the circumflex artery while the dogs were running on a treadmill triggered ventricular fibrillation (VF) in nearly two thirds of the animals[47,119]. The incidence of VF could be reduced by right VNS[120] and the protective effect of VNS was abolished by administration of atropine, a blocker of muscarinic receptors.

[H2] Acute effect of cholinergic signalling on ventricular arrhythmias Depolarization of postganglionic vagal cholinergic neurons **[G]** causes the release of acetylcholine, which acts on muscarinic receptors. In sinoatrial node cells, activation of M2 muscarinic acetylcholine receptors leads to hyperpolarization via G_k stimulation of the acetylcholine-activated inward-rectifier K^+ current (I_{KACh}) and to a slowing in the rate of diastolic depolarization via G_i-dependent inhibition of adenylate cyclase, which reduces cAMP levels and the hyperpolarization-activated Na^+ inward current (or funny current; I_f) and reduces PKA-dependent

phosphorylation of L-type Ca^{2+} channels Overall, these effects produce an abrupt reduction in the heart rate[121]. Heart rate reduction itself can be beneficial because it lowers the cardiac metabolic demand while improving diastolic coronary perfusion time and oxygen delivery. Some arrhythmogenic mechanisms, such as instability of intracellular Ca^{2+} handling that causes electrical alternans **[G]**, are also more prevalent at high heart rates[122]. Several studies have demonstrated that the antiarrhythmic action of VNS can be blunted by pacing the heart at a constant rate[114,118,123-125], although more recent data have demonstrated that an antifibrillatory effect of VNS still persists during cardiac pacing at a fixed heart rate[126,127].

Stimulation of the M2muscarinic receptor in the ventricle is also coupled to G_i and G_k signalling cascades. G_i-dependent reduction in I_{CaL} together with decreases in SERCA2 activity (regulated by phospholamban) prevent Ca^{2+} overload and the generation of DADs G_i-dependent reduction in I_{Ks} can also prolong the ventricular action potential and refractory period. In addition, the vagus nerve can have an indirect arrhythmogenic effect via 'accentuated antagonism' of adrenergic signalling. M2 Muscarinic receptor activation of G_k leading to increased I_{KACh} is proposed to be the main mechanism mediating cholinergic accentuated antagonism of β-adrenergic receptors[128]. Other investigators have proposed that accentuated antagonism occurs at the levels of cAMP, whereby M2 muscarinic receptor stimulation of endothelial NO synthase (eNOS) [129] can increase the activity of the cGMP-dependent phosphodiesterase PDE2A to reduce adrenergic-mediated cAMP signalling[130]. However, other studies demonstrate that accentuated antagonism of heart rate and contractility by the vagus nerve is preserved despite pharmacological inhibition of this pathway[131] or gene knock out of *Nos3*, which encodes eNOS[132].

Of note, voltage mapping experiments in isolated rabbit hearts demonstrate a prolongation of the ventricular refractory period, flattening of the APD restitution curve and a rise in the VF threshold in response to both left or right VNS[133] or to cholinergic receptor stimulation [127], in the absence of β-adrenergic receptor stimulation. Prolongation of the refractory period during increased vagal efferent activity is also observed independently of heart rate in clinical electrophysiological studies[134].

NO generated by nNOS also has an important role in the antifibrillatory effect of cholinergic stimulation[126]. nNOS can alter parasympathetic neurotransmission in terms of vagal control of the heart rate. Pharmacological manipulation of NO signalling[135,136] or knock out of *Nos1* (which encodes nNOS) blunts the response to VNS but does not alter the bradycardia triggered by muscarinic receptor stimulation, indicating that the site of action of the nNOS-derived NO is pre-synaptic. Moreover, NO induces an increase in acetylcholine release in vagal neurons, mediated by augmenting cGMP levels, which inhibits PDE3, thereby leading to an increase in cAMP levels and PKA-dependent phosphorylation of N-type Ca^{2+} channels[131]

In rabbits, the antifibrillatory effect of left or right VNS *in vitro* persisted despite atropine administration, but could be prevented by NOS inhibition[126,137], suggesting that NO is released as a co-transmitter from

parasympathetic efferent or afferent fibres. Whereas NOS inhibition prevented the effect of VNS on APD restitution, a shortening of the refractory period still persisted. By contrast, nNOS inhibition reduced the ventricular refractory period in rats with through silencing of dorsal vagal motor nucleus preganglionic neurons[139].

Other studies show that the antiarrhythmic effects of cholinergic receptor stimulation are co-dependent on neuronal NO production and muscarinic receptor stimulation. In isolated rat hearts, an acetylcholine analogue prolonged APD, flattened the APD restitution curve and increased the VF threshold[127]. This increase in the VF threshold was abolished by specific and non-specific nNOS inhibition or by downstream inhibition of soluble guanylyl cyclase. An increase in NO metabolites was also observed, which was prevented by inhibition of nicotinic receptors on postganglionic cholinergic neurons. The antifibrillatory effect of both the acetylcholine analogue and a NO donor was completely abolished by atropine administration, suggesting that nNOS-derived NO modulates vagal neurotransmission[136]. The critical role of muscarinic receptors in the antiarrhythmic effect of VNS is also supported by recent studies of VNS in ischaemia–reperfusion injury[140], as well as by classical *in vivo* studies that used atropine to block the antiarrhythmic effect of VNS [115,117,120,141,142]. The most likely mechanistic explanation for these observations is that nNOS-derived NO from parasympathetic ganglia facilitates acetylcholine release from sites of ganglionic projections. Whether NO also acts as a co-transmitter might depend on the density of innervation and the expression or activity of nNOS, given that NO is a highly diffusible and reactive signalling molecule[143].

[H2] Chronic effect of cholinergic signalling on the arrhythmogenic substrate. A heterogeneous, fibrotic MI scar is an important substrate for the formation of re-entry and VT. Inflammatory pathways have a key role in fibrosis[144], scar formation and hypertrophy[145], and inflammatory mediators such as IL-1 can be directly arrhythmogenic[146]. VNS in rats during ischaemia–reperfusion reduces the infarct size, the number of inflammatory cells in the area at risk of injury and the levels of circulating inflammatory cytokines via a nicotinic receptor pathway[147]. Chronic VNS in a dog model of HF also normalizes the levels of IL-6 and TNF[148], and reduces plasma levels of angiotensin II [149], a potent pro-fibrotic mediator. Conduction velocity through scar tissue and in the normal myocardium critically depends on the expression and function of gap junction proteins. After MI, chronic VNS preserved connexin 43 phosphorylation and reduced the prevalence of spontaneous VT in rats[150], and normalized connexin 43 levels in a dog model[151]. The chronic effects of VNS on myocardial substrate remodelling might have an important role in its antiarrhythmic effect.

Cardiac gene transfer of *Nos1* targeted at the right atrium via a pericardial approach has been tested in a small animal model and shown to increase vagal nerve activity and reduce heart rate[152] and mortality after MI[5]. However, targeting cholinergic ganglia in the ventricle with the use of this approach as an

antiarrhythmic therapy has not been tested yet, although gene transfer directly into the cervical vagus nerve can increase cardiac baroreflex sensitivity in pigs[153]. Of interest, exercise training improves baroreflex control of heart rate and reduces susceptibility to arrhythmia[154]. Exercise training can also upregulate cardiac vagal nNOS activity, promote vagal transmission[155] and also decrease sympathetic responses[156], suggesting that cholinergic signalling is part of an endogenous protective pathway associated with the beneficial effects of physical training.

[H1] Neuromodulation in ventricular arrhythmia

Primary percutaneous coronary intervention and improved medical therapy have revolutionised the treatment of patients with MI; however, subsequent sudden cardiac death remains an important clinical problem. Enhanced sympathetic nerve activity is typical in patients with previous MI or with HF, and is a key contributor to the increase in arrhythmogenicity observed in these patients. These patients can present with VT that is recurrent or incessant, resulting in haemodynamic instability and high mortality and in patients with an implantable cardioverter–defibrillator (ICD), requiring multiple ICD shocks[162].

The potential for VNS to provide an effective neuromodulatory approach for the treatment of ventricular arrhythmia is well founded in mechanistic experimental studies, as described in the previous section, but has yet to be convincingly translated into clinical practice. Clinical studies on VNS for the treatment of advanced HF have been disappointing and have not targeted reduction of ventricular arrhythmia as an end point. Pharmacological approaches for the treatment of ventricular arrhythmia in HF, such as cholinesterase inhibition[157], also have limitations. Therefore, the sympatho-adrenal axis has been the principal target for drug treatment with β-blockers and angiotensin-converting enzyme inhibitors in patients with MI or with HF, with both drug therapies inducing a significant reduction in morbidity and mortality[158-161].

Current secondary prevention VT management strategies include the use of antiarrhythmic drugs and catheter ablation[163], but durable freedom from recurrent VT remains suboptimal[164]. While repeat ablation procedures remain as a treatment option for VT, additional therapies targeting the sympatho-adrenal neural axis have shown promise and have moved from bench to bedside (Figure 1). Experimental studies have supported this paradigm shift in therapeutic approach.

[H2] Cardiac sympathetic denervation. The anterior wall of the left ventricle is innervated by bilateral stellate ganglia and their stimulation results in noradrenaline release and electrophysiological changes that increase the risk of ventricular arrhythmia[165,166]. The stellate ganglia in patients with cardiomyopathy and ventricular arrhythmia are characterized by hypertrophy, inflammation, remodelling and oxidative stress[98]. The

remodelling process after myocardial injury takes place at multiple levels of the cardiac neural axis and also affects the intrinsic cardiac nervous system (ICNS), with heterogeneity in the afferent signal from the scar and border zone regions[36]. The deleterious effects of cardiac neural axis remodelling on cardiac electrophysiology in a pig model could be reversed with bilateral stellectomy (a procedure known in patients as cardiac sympathetic denervation, CSD), resulting in a reduction in VT inducibility and a small correction in activation recovery interval (a surrogate for APD)[167].

The clinical efficacy of CSD was first demonstrated in the context of LQTS[168,169] and catecholaminergic polymorphic VT[170]. In the largest study in patients with LQTS who were undergoing left CSD, >50% of previously symptomatic patients became asymptomatic and in the remaining patients, the annual rate of cardiac events decreased by up to 91%[171]. In patients with HF and with refractory ventricular arrhythmia that did not respond to catheter ablation, a large, contemporary study reported the efficacy of left or bilateral CSD[172,173]. This approach resulted in a >80% reduction in the number of ICD shocks after the procedure, with bilateral CSD being more effective than left CSD alone (Figure 5c).

[H2] *Thoracic epidural anaesthesia*. Thoracic epidural anaesthesia (TEA) has also demonstrated therapeutic efficacy in this patient population, and can be performed as an emergency procedure at the bedside[174]. The mechanisms of TEA have been studied in dog models[175] and involve attenuation of afferent and efferent cardiac neurotransmission with prolongation of the myocardial refractory period[176]. With this approach, patients can be safely bridged to more definitive therapies. The largest, multicentre clinical study on TEA included 11 patients with VT storm **[G]** [177]. All patients tolerated the TEA without haemodynamic compromise, with >50% of patients experiencing a complete or partial response to the therapy. One of the major limitations of TEA is that this approach cannot be performed with concomitant use of antiplatelet medications or anticoagulants; therefore, an alternative percutaneous strategy for patients requiring antithrombotic therapy is ultrasound or fluoroscopy-guided, cervico-thoracic stellate ganglion block. A meta-analysis of studies on stellate ganglion block demonstrated a significant decrease in ventricular arrhythmias and the number of ICD shocks after the procedure that was independent of the underlying aetiology[178].

[H2] *Renal denervation.* Mechanistic studies have shown that renal denervation can prevent noradrenaline spillover, which is extenuated by abnormal sympathetic afferent activity[179] and influences ventricular electrophysiology[180,181]. Small-scale clinical trials have reported that bilateral renal denervation is an effective therapy for refractory ventricular arrhythmia[182-184]. This approach requires optimization in terms of denervation site and energy delivery before larger studies are undertaken to confirm its efficacy, in view of the disappointing results of the SYMPLICITY HTN-3 trial of renal denervation in drug resistant hypertension[185], although it is worth noting that more recent randomized, sham controlled trials in patients either taking (SPYRAL HTN-ON trial) or not taking antihypertensive medication (SPYRAL HTN-OFF MED trial[186] and RADIANCE SOLO trial) seem more promising.

[H2] *Approaches at experimental stages targeting the sympatho-adrenal neural axis.* Other emerging targets for neuromodulation of the sympatho-adrenal neural axis include distal ligament of Marshall ablation and optogenetic manipulation of stellate ganglia physiology. The distal ligament of Marshall serves as a conduit between the left stellate ganglia and the ventricular myocardium. In a dog model of MI, ablation at this site prevented ventricular arrhythmia[187]. A study in dogs published in 2017 utilized an adenovirus associated vector to deliver to stellate ganglia the light-activated proton pump archaerhodopsin T (ArchT), which produces inhibitory currents when stimulated with green LED light[188]. The vector demonstrated specificity for the stellate ganglia and stimulation with light suppressed sympathetic activity, as evidenced by prolongation of ventricular APD and effective refractory period, improved heart rate variability and protection against ischaemia-induced ventricular arrhythmia.

Sympathetic neuromodulation can also be achieved at sites far removed from the cardio–renal axis. Deep brain stimulation of the periaqueductal grey is used for the treatment of chronic pain, but can alter the blood pressure depending on location of the stimulation electrode[189,190], heart rate variability[190] and baroreflex sensitivity by influencing sympathetic outflow[191]. Spinal cord stimulation at T1–T3 has also been shown to reduce cardiac sympathetic drive, and continuous spinal cord stimulation improved symptoms in a small group of patients with severe symptomatic HF[192]. Furthermore, an animal model of spinal cord stimulation demonstrated suppression of left stellate ganglia activity and post-MI ventricular arrhythmia[193]. However, the randomized, single-blind DEFEAT-HF trial[194] did not provide evidence of any meaningful change in clinical outcomes in patients with HF.

[H2] *Vagus nerve stimulation.* As discussed above, direct cervical VNS has demonstrated substantial promise in animal models of post-MI ventricular arrhythmia, which might be related to the observation that acetylcholine levels and parasympathetic neuronal pathways are preserved in the infarct border zone, enabling electrical stabilization after VNS[195]. However, after a promising proof-of-concept study[198], subsequent clinical trials of VNS in advanced HF (including NECTAR-HF and INOVATE-HF) had disappointing results[196,197]. Although arrhythmia end points were not evaluated in these trials, HF symptoms did not improve. The ANTHEM-HF study[199200] compared right or left VNS in a small group of patients and demonstrated improvements in symptoms and echocardiographic parameters at both 6 months and 12 months of follow up compared with baseline, although the trial did not include a control group. A substudy of Holter recordings from this study also demonstrated a reduction in the incidence of T-wave alternans and non-sustained VT, as well as favourable effects on heart rate turbulence, a marker of baroreceptor function[201]. On the back of these results, a large, randomized, controlled trial of right VNS with the use of the same system is now underway (ANTHEM-HF PIVOTAL trial, NCT03425422). Of note, these trials used a variety of stimulation parameters and techniques, which might influence the efficacy of the approach[110,202]. How this and other aspects of device and trial design might have led to neuromodulation being ‘lost in

translation' have been discussed extensively[203]. Given the scientific basis for VNS and the opportunities to improve stimulation parameters, programming and hardware, there is scope to be optimistic for this approach, especially given the long record of safety and efficacy of VNS in the treatment of >100,000 patients with drug-resistant epilepsy.

[H1] The ANS and atrial fibrillation

Atrial fibrillation (AF) is the most common pathological cardiac arrhythmia, and is associated with significant morbidity and mortality. AF produces a cascade of structural and electrical remodelling in the atria including chamber dilatation and wall fibrosis[204] such that further AF becomes increasingly more likely and restoration of sinus rhythm more difficult. This phenomenon is encompassed by the well-known adage that 'AF begets AF'[205]. The seminal work of Haissaguerre and colleagues defined the role of rapid firing from the pulmonary veins in initiating paroxysmal AF[206]. Of note, the pulmonary veins contain a variety of myocytes capable of generating ectopic activity, which can then reach the atria and induce AF[207]. The pulmonary veins might also provide a non-excitable gap to support re-entry, and are also highly innervated[208]. The atrial ICNS is an extensive, interconnected epicardial network of ganglionic plexi, nerve axons and interconnecting neurons. These structures are embedded within the epicardial fat pads, except the ligament of Marshall, which runs between the left atrial appendage (LAA) and left pulmonary vein antrum. These structures vary in size from those containing a few neurons to some with >400 neurons[209,210].

The vagal nerve influence on AF has been known historically and VNS or acetylcholine administration can lead to AF[211]. Furthermore, some forms of paroxysmal AF are related to elevated vagal tone[212]. Of note, exercise training has been associated with an increased risk of AF[213], although exercise has a variety of beneficial effects on overall cardiovascular health. This relationship seems to become stronger with age[214] and confers an overall fivefold higher risk of AF in athletes compared with sedentary individuals[215]. AF in athletes also occurs during periods of high vagal tone, such as at night, at cessation of exercise and in the post-prandial period[216].

The electrophysiological effects underpinning these observations include spatially heterogeneous atrial refractoriness[217,218], and shortening of atrial propagation wavelength[219] with cholinergic activation. These effects might be mediated at least in part by increasing I_{KAch} in a spatially heterogeneous manner, thereby promoting re-entry. Direct recording of stellate ganglia and vagus nerve activity in animals enables correlation with temporal changes in nerve activity and AF initiation[220] Simultaneous activation of both branches of the ANS might be required to initiate AF. This requirement would be unsurprising given that the effects of muscarinic receptor activation are exacerbated during background β-adrenergic receptor

stimulation, via accentuated antagonism. Direct stimulation of ganglionic plexi associated with the left atrial fat pads can lower the threshold for induction of AF or precipitate paroxysms of AF[221]. Acetylcholine injection into ganglionic plexi results in firing from adjacent pulmonary veins[222] and sustained AF. Furthermore, the cellular electrophysiology of the pulmonary vein muscular sleeve has enhanced sensitivity to autonomic stimuli, which allows a shorter APD than in the surrounding atrial myocardium[223,224].

Experimental studies with autonomic stimulation of isolated pulmonary veins demonstrate induction of early afterdepolarizations and triggered activity similar to those seen in patients with paroxysmal AF[223]. High-frequency stimulation in the vein of Marshall can also trigger AF and atrioventricular nodal conduction slowing, demonstrating a communication between ganglionic plexi in the vein of Marshall and the right inferior ganglionic plexi (which controls the atrioventricular node). Such a response was eliminated after ethanol infusion in the vein of Marshall[225], which produced regional parasympathetic denervation[226]. Targeting the ligament of Marshall has traditionally been performed during epicardial surgical AF ablation (the maze procedure, also known as Cox maze III), and percutaneous ethanol infusion in the vein of Marshall is currently being assessed in a randomized clinical trial[227].

Although electrical and structural remodelling of the atria in AF is widely appreciated, comparatively little is known about neural remodelling and how it may contribute to AF. A heterogeneous increase in atrial sympathetic nerve density has been observed both in animal models of AF[228,229] and in patients with AF who were undergoing cardiac surgery[230] Low-level stimulation of the cervical vagus nerve, either directly or transcutaneously via the tragus, is thought to silence ganglionic plexi, reduce systemic markers of inflammation and AF inducibility[231] and decrease the occurrence of paroxysmal AF and post-operative AF after cardiac surgery[232]. Inflammation has an important role in the pathogenesis of AF and might have a role in neural, myocyte and fibrotic remodelling, whereby inflammation and remodelling can perpetuate each other and maintain AF[231]. Ganglionic plexi and the ICNS function independently or in response to higher levels of the cardiac neural axis. Ganglionic plexi and the ICNS can form a final effector pathway in the autonomic, pro-fibrillatory influence on the atria, which makes these structures an attractive, anatomically accessible target for therapy in patients with AF. However, the basic physiology of this complex neural network is still being elucidated.

[H1] Neuromodulation in atrial fibrillation

The current methodology for percutaneous catheter ablation in AF involves wide, circumferential pulmonary vein isolation (PVI) with either radiofrequency or cryoablation. The current approach for antral PVI inevitably also targets the anatomical locations of the ganglionic plexi, and the physiological effects include

bradycardia during ablation[233] and alterations in heart rate variability in the long term[234]. A single, left atrial ganglionic plexus is thought to innervate one of the pulmonary veins as well the surrounding atrial myocardium[209,210] (Figure 4a). Autonomic denervation is seen frequently after PVI[235-237] and observational studies have reported a decrease in AF recurrence when this denervation occurs[238-240]. Complete PVI might not be required to maintain sinus rhythm, also supporting the observations of autonomic denervation [238,241-243]. The conventional lesion set after PVI transects the anatomical location of three of the major ganglionic plexi sites; therefore, interruptions of these projections might contribute to procedural success[244]. However, the specific neural elements that are responsible for the therapeutic effect of PVI remain to be determined.

Small, randomized, controlled trials have supported the role of ganglionic plexi ablation in addition to PVI[239,240] or as an isolated strategy[245] for the treatment of AF. Addition of ganglionic plexi ablation improved success rates by 25%, whereas ganglionated plexi ablation alone in paroxysmal AF or persistent AF was successful in 71–86% of the patients. The largest randomized trial on the effect of ganglionated plexi ablation combined with PVI in 242 patients with paroxysmal AF demonstrated a significant improvement in AF and atrial tachycardia free survival in the group receiving ganglionated plexi ablation and PVI compared with the groups receiving PVI alone or ganglionated plexi ablation alone[246]. A similar approach in patients with persistent AF showed that PVI plus ganglionated plexi ablation was superior to PVI plus linear ablation, with the additional benefit of a decrease in left atrial tachycardias[247]. However, in patients with drug-refractory, long-standing, persistent AF, ganglionated plexi ablation alone resulted in worse outcomes than PVI[248].

Some data correlate the areas of endocardial complex fractionated atrial electrograms (CFAEs) in AF with the presence of ganglionated plexi[249-251], although imaging accurately the anatomical location of ganglionated plexi during an ablation procedure is currently not possible. The results of the large, randomized, STAR-AF II trial[252] assessing PVI alone, PVI plus left atrial linear ablation and PVI plus ablation of CFAE sites for the treatment of persistent AF should also be considered. In this study, which has influenced clinical practice worldwide, PVI alone was superior to the other approaches. Ganglionated plexi activity might be most relevant in paroxysmal AF, while becoming less important with progression to persistent AF and the development of atrial remodelling and fibrosis[231].

Another approach to localize the ICNS or ganglionated plexi is based on the response to endocardial high frequency (20Hz) stimulation while looking for inhibition of atrioventricular nodal conduction or increases in the R-R interval by 50% during AF[240]. Although a single-centre study reported improvements in outcome with the use of high frequency stimulation to target ganglionated plexi ablation, more ablation was undertaken in this group, which might have influenced these results. Experimental observations in animals[253] and humans[254] have suggested that targeting ganglionated plexi with inputs to pulmonary veins might produce better results than high frequency stimulation. The electrophysiological approach based on identification of

vagal responses also lack sensitivity[231]. Atrial ganglionated plexi contain a complex network of efferent, afferent and inter-neuronal populations expressing a variety of neurotransmitters. Disruption of atrial cholinergic neurons can also have a detrimental influence on ventricular electrophysiology because cholinergic neurons run alongside sympathetic fibres from the atria to the ventricles[255], particularly after acute MI[256].

In addition, a complex series of interactions exists between the extrinsic cardiac nervous system (ECNS) and the ICNS[257], which might enable the exploration of alternative neuromodulation therapies. The ganglionated plexi integrate autonomic inputs with multiple interconnections with a final common pathway to the sinus and atrioventricular nodes via the right anterior and inferior ganglionated plexi. Ablation of ganglionated plexi between the ECNS and ICNS leads to an increase in AF burden, suggesting the presence of inhibitory effect to preserve sinus rhythm[258]. The reduction in vagal tone with ageing might, therefore, contribute to loss of this tonic suppression of ganglionated plexi activity and the corresponding increase in AF prevalence[259]. This interaction has been translated clinically into bioelectronic neuromodulation with low level VNS, a technique that has shown efficacy in epilepsy[260] and in early clinical trials in HF[198]. Applying low level VNS without inducing bradycardia influenced downstream atrial electrophysiology, with an increase in the effective refractory period in the atrium and pulmonary veins, suppression of AF inducibility and a decrease in the duration of acetylcholine-induced AF[261,262].

Direct recordings of ganglionated plexi activity during VNS demonstrate a decrease in the frequency and amplitude of discharges[262]. Surgical VNS applied extra-epicardially, rostral to the heart, has been studied in a randomized control trial in patients undergoing cardiac surgery[263]. Stimulation below the threshold for bradycardia resulted in a significant reduction in post-operative AF (12% versus 36%) and the levels of circulating inflammatory cytokines. Interestingly, although VNS was applied only during the intensive care period after cardiac surgery (72 h), efficacy of AF suppression was maintained during the 1 month of follow-up. This memory effect of VNS has been previously reported[31,264] and might indicate future implications for the application of novel neuromodulatory therapies. Surgical VNS is feasible in the context of patients undergoing cardiac surgery, but might not be applicable to the large number of patients with AF.

Low-level tragus stimulation (LLTS) targets the auricular branch of the vagus nerve and has been shown to result in brainstem evoked potentials **[G]** in humans[265] and to suppress pacing-induced AF in experimental models[266]. LLTS restored normal anterior right ganglionated plexi activity following rapid pacing[266]. In patients with paroxysmal AF referred for ablation, LLTS decreased rapid-pacing-induced AF duration and plasma TNF levels[232]. Further studies are required to evaluate these and other novel approaches for the prevention and treatment of AF, including botulinum toxin injection into epicardial fat pads[267] and renal denervation[268]. Of note, in a study in >700 patients receiving orthotopic heart transplants, in which the heart

is completely decentralised, AF was only observed after the post-operative recovery period in the presence of rejection or vasculopathy[269].

[H1] Discovery of novel neuronal targets

With the advent of high throughput sequencing, RNA sequencing provides the opportunity to identify key transcripts (for hypothesis testing) that might encode proteins involved in abnormal neurotransmission. This technique can be applied to whole tissue but can also be performed at a single cell level, making RNA sequencing ideally suited for studying neural remodelling of autonomic efferent and afferent nerves once isolated. For example, emerging evidence suggests that in stellate ganglia from rats with a sympathetic phenotype of hyperactivity, a significant number of differentially expressed transcripts compared with healthy rats are linked to impaired regulation of intracellular Ca^{2+} homeostasis and exocytosis[270,271] (Figure 6). Moreover, these transcripts are conserved in human stellate neurons, thereby providing wider physiological context. Bardsley et al. reported differential expression of several transcripts that were validated using quantitative reverse transcription PCR (qRT-PCR)[270]. Several gene ontology groups were identified with network and enrichment analyses. The identified gene ontology families and functional pathways included transcripts related to extracellular ligand-gated ion channel activity, in particular glutamatergic and dopaminergic signalling pathways linked to modulating Ca^{2+} balance. PDE activity was also altered[270], supporting previous reports indicating that impaired cyclic nucleotide signalling is coupled to sympathetic dysautonomia[272].

Although changes in transcript levels do not directly equate to changes in protein levels, nor do they indicate protein–protein interactions, transcriptome changes nevertheless provide a framework to test the functional role of lead candidate genes in the regulation of physiological processes. For example, cells host several isoforms of PDEs, enzymes with dual-specificity for cAMP and cGMP that are involved in maintaining the balance of cAMP and cGMP levels. In stellate ganglia from rats with sympathetic hyperactivity, expression of *Pde2a* and *Pde11a* was decreased whereas expression of *Pde6b* was increased compared with stellate ganglia from normal rats. However, closer examination in diseased rats and human stellate tissue reveals that PDE2A protein and activity levels are increased in conditions of sympathetic hyperactivity[273]. Moreover, if PDE2A is directly up-regulated by overexpression with an adenovirus in healthy neurons, agonist-activated cGMP levels decrease due to increased PDE2A hydrolytic activity, thereby mimicking the diseased state. The decreased cGMP levels are associated with increased Ca^{2+} currents and Ca^{2+} transients, leading to greater release of neuronal noradrenaline[274]. Interestingly, in diseased neurons, pharmacological inhibition of PDE2A or gene transfer of the dominant negative form of PDE2A restores the inhibitory action of receptor-coupled cGMP activators to decrease intracellular Ca^{2+} and neurotransmission[273].

What is not fully established is whether the identified transcripts associated with impaired sympathetic function are fully conserved across mammalian genomes. Most of the lead candidate transcripts identified in the study by Bardsley et al.[270] ~~are~~ present in the mouse stellate ganglia and, interestingly, a significant sex difference in expression levels has been observed[275], which might need to be considered when studying other species. Although transcriptomes provide a molecular 'road map' for further studies, transcriptomics only provides a statistical association to promote a direction of investigation. Validation of the transcript at the protein level and, more importantly, showing that proteins encode function (physiology) and are conserved in human tissue, will be essential to establish target discovery and clinical utility.

Even though the use of transcriptomics is 'hypothesis neutral', this technique can nevertheless reveal surprising associations previously missed with the use of conventional pharmacological approaches. Bardsley et al. observed low-level expression of genes encoding pre-synaptic β-adrenergic receptors in the rat and showed with the use of qRT-PCR that these receptors were conserved in human stellate neurons[40]. Early studies in animal models had reported the presence of β-adrenergic receptors in postganglionic sympathetic neurons[276] and suggested a possible role for these receptors in the regulation of noradrenaline release during nerve stimulation, the so called 'adrenaline hypothesis' of hypertension. However, the presence of these receptors in human tissue and the precise signalling pathway underpinning this hypothesis was not established until recently. Using a combination of RNA sequencing, immunocytochemistry, Forster resonance energy transfer imaging to assess cAMP–PKA activity, and measurements of intracellular Ca^{2+} levels, Bardsley et al. identified a functional Ca^{2+}-dependent exocytosis that was predominately activated via a β_2-adrenergic receptor stimulation of the cAMP–PKA pathway in diseased neurons only[40] (Figure 7). Increases in the levels of phenylethanolamine-N-methyltransferase led to neurotransmitter switching in preference for the synthesis of adrenaline in rat and human stellate neurons[40] (Figure 7). Of interest, the study also reported an increased evoked release of both noradrenaline and adrenaline in diseased neurons from pre-hypertensive rats, establishing evidence that catecholamine levels were raised before the overt signs of hypertension and adding weight to the model that pre-synaptic release of noradrenaline and adrenaline might further drive sympathetic transmission through positive feedback stimulation of neuronal β-adrenergic receptors. Together with circulating catecholamines, neuronal release of catecholamines might be a powerful drive of cardiac postsynaptic excitability[277,278] (Figure 7).

The mechanism driving enhanced cardiac sympathetic transmission in cardiovascular disease is not fully established. What is clear is that the autonomic phenotype can precede the overt clinical signs of the disease[279-281] and reside in both the peripheral and central part of the cardiac neural axis[282]. Several research groups have argued that oxidative stress is a key hallmark of sympathetic dysautonomia, and impairment of the NO–cGMP pathway has been implicated[100]. Under normal physiological conditions, NO–cGMP provides an inhibitory action on sympathetic transmission by activating cGMP-dependent stimulations of PDE2A to

decrease cAMP-coupled phosphorylation of neuronal Ca^{2+} channels[283-285]. Overexpression of *Nos1* with a noradrenergic-specific promoter can rescue impaired NO–cGMP signalling and restore Ca^{2+}-dependent exocytosis in a rat model of sympathetic hyperactivity[286,287].

Another clue regarding the importance of the NO–cGMP pathway has come from the 'top down' approach of genome wide association studies (GWAS), rather than the 'bottom up' approach of transcriptomics. Modulation of the NO pathway is strongly influenced by the coupling of the nNOS adaptor protein CAPON (also known as NOS1AP)[288]. The potential importance of CAPON was first highlighted by the identification by GWAS of single nucleotide polymorphisms in *NOS1AP* associated with QT interval variation[289] and with sudden cardiac death[290]. Polymorphisms in *NOS1AP* have also been identified as risk modifiers for arrhythmic events and sudden cardiac death in patients with LQTS type 1[291]. Overexpression of *NOS1AP* in cardiomyocytes led to shortening of the QT interval via a putative pathway in which CAPON–nNOS induced a cGMP-dependent inhibition of I_{CaL} and activation of I_{Kr}, causing a faster repolarization of the APD that resulted in restoration of the QT interval[292]. Of interest, one of the major triggers of sudden death in patients with certain types of LQTS is a sympathetic surge that is experienced during strenuous exercise and psychological stress[293]. CAPON was first discovered in the brain[294], but is also present in peripheral autonomic ganglia[288]. Overexpression of this adaptor protein in sympathetic neurons decreases the N-type Ca^{2+} current (I_{CaN}), the Ca^{2+} transient and subsequent neurotransmission via a cGMP-dependent pathway[288]. The functional significance of this pathway in modulating arrhythmia is not firmly established, but mutations in CAPON might facilitate abnormal sympathetic transmission and trigger afterdepolarizations (Figure 8).

[H1] Conclusions

Returning to the three-level neural hierarchical model of the ANS shown in Figure 2b, and the feedback loops that exist within and between levels, several things become clear. First, this model is an oversimplification of a complex neural network, and how the ANS is remodelled with disease is poorly understood. Moreover, the tools we have to interact with the system, in particular neural stimulation, surgical resection and ablation, are also crude. None of these interventions solely target efferent or afferent neurons from a single branch of the ANS. That said, we remain optimistic about the research community's ability to unravel this complexity. Techniques that have advanced neuroscience in the last decade, such as optogenetics, genetic manipulation, gene editing and CLARITY **[G]**, combined with more traditional neurophysiological techniques are likely to make great inroads. However, prematurely racing towards clinical trials with little understanding of what we are doing risks damaging the entire field. We need to be very careful that we do not make the same mistakes seen in other medical fields throughout history. It

would be a shame if intervention for arrhythmia on the 'heart's little brain' became for cardiology what lobotomy for schizophrenia became for neurosurgery.

Of note, some of the more successful interventions to date for ventricular arrhythmias target the stellate ganglia. The stellate ganglion is at least a tractable target, in that it is an important relay station for cardiac afferents and contains the cell bodies of mainly efferent post-ganglionic neurons communicating directly with the myocardium. The stellate ganglion is also easily accessible, at least when compared with the brainstem and other level 1 structures in the neural hierarchy. When assessing interventions (particularly stimulation parameters and patterns), these interventions should be systematically and thoroughly tested in representative animal models of disease before trialling in humans.

It should also be considered that patients will already be receiving the current standard of care in terms of pharmacotherapy. This therapy can include β-blockers and medications targeting the renin–angiotensin–aldosterone system, which also influence the ANS. Therefore, these medications are likely to influence the efficacy of approaches to neuromodulation. Use of β-blockers, for example, might uncover a more prominent role for other adrenergic receptors or indeed co-transmitter receptors in the ongoing disease process. We suggest that new therapies are also tested in animal disease models receiving the same therapeutic treatment as patients with the disease to see whether the treatment influences the effectiveness of the new approach. The influence of sex differences should also be considered.

Although cardiac sympathetic denervation has shown significant clinical utility with a low complication rate, opportunities exist to refine our manipulation of this site (Figure 5). In this context, we feel that transcriptomics and proteomics of the stellate ganglia, and other neuronal populations might help to identify how the ANS remodels during disease, and in so doing, provide a molecular framework and road map to test the functional significance of these pathways. This approach might also, to some extent, help to validate how close different animal models are to their human disease counterparts, and potentially to identify new therapeutic targets. Finally, being able to differentiate human stem cells into cardiac cells and sympathetic neurons from patients with genetic autonomic phenotypes, and then study these cells *in vitro,* might aid us in understanding human pathophysiology.

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Author contributions

N.H. and D.J.P. conceived the Review idea. All authors researched the data for the article, provided substantial contributions to discussions of its content, wrote the article and undertook review and/or editing of the manuscript before submission.

Competing interests

The authors declare no competing interests.

Key points

- Many primary cardiovascular diseases, such as hypertension, acute myocardial infarction and heart failure, are also diseases of the autonomic nervous system.
- Sympathetic over activity and vagal impairment are a powerful negative prognostic indicator for morbidity and mortality associated with arrhythmia and sudden cardiac death.
- Emerging evidence suggests neuromodulation therapy might provide important clinical utility in the management and prevention of lethal arrhythmia.
- Neuromodulation device therapy has yielded conflicting and disappointing results in clinical trials, which might be related to stimulation parameters and/or lack of site-specific targeting and appreciation of the complex neural circuitry driving postsynaptic excitability.
- Surgical resection or ablation of specific ganglion, in particular the stellate ganglion, has produced encouraging therapeutic benefits in patients with sympathetic hyperactivity who are prone to arrhythmia.

- Understanding the relationship between neural circuitry and the molecular pathways underpinning abnormal neurotransmission to cardiac electrophysiology is essential to improve neuromodulation therapy.

Figure 1 | **Neuromodulation targets for treating cardiac arrhythmia**. Schematic representation of the extrinsic and intrinsic autonomic nervous system and the different targets for autonomic nervous system modulation in the treatment of cardiac arrhythmias. AF, atrial fibrillation; MI, myocardial infarction; PVI, pulmonary vein isolation; VF, ventricular fibrillation; VT, ventricular tachycardia. Modified from Krul et al[4].

Figure 2 | **The cardio–neural hierarchy a** | Schema of the cardiac nervous system. **b** | Simplified construct of neurohumoral control and functional organization of cardiac innervation. β_1, β_1-adrenergic receptor; C, cervical; DRG, dorsal root ganglion; G_i, inhibitory G protein; G_s, stimulatory G protein; ICNS, intrinsic cardiac nervous system; L, lumbar; M2, muscarinic acetylcholine receptor M2; T, thoracic. Modified from Shivkumar et al[9].

Figure 3 | **Cardiac autonomic innervation in humans**. Diagrams showing the complexity of the human autonomic cardiac nervous system, with great arterial bifurcations viewed from the ventral (part **a**) and dorsal (part **b**) aspects. The sympathetic, parasympathetic vagal, and mixed nerves are coloured *orange*, *green*, and *purple*, respectively. *Black stars*, *squares*, and *circles* indicate the inlet/outlet of the cardiac nerve/branches from the arterial part of the cardiac hilum itself, and venous parts of the cardiac hilum. From Kawashima[297]. Redrawn with permission of Elsevier.

Figure 4 | **Cardiac autonomic innervation a** |Posterior view of the human heart and major vessels illustrating the locations of posterior atrial and ventricular ganglionated plexi (GP). Note the mediastinal nerves coursing adjacent to the aortic root and joining the two superior atrial ganglionated plexuses. Positions of the superior vena cava, inferior vena cava, right ventricle (RV), left atrium (LA) and left ventricle (LV) are shown. **b** | Confocal image of intrinsic neurons in mouse heart labelled with multicolour adeno-associated viral technique. **c** | Embryonic day 18.5 mouse heart stained for myocardium (red) and tyrosine hydroxylase (green) optically cleared using 3-Disco, showing that before birth, the sympathetic innervation has already formed a dense network on the epicardial surface of the ventricle and is starting to penetrate the myocardial wall. Panel a is from Armour et al[209]. Panel b is from Rajendran et al[36] with permission. Panel c is Courtesy of Dr Juanjuan Zhao and Dr Mathilda Mommersteeg, Department of Physiology, Anatomy & Genetics, University of Oxford.

Figure 5 | **The autonomic nervous system in cardiac a** | Cardiac disease progression. Distribution of autonomic nerves in the heart and disease progression from healthy state to heart failure, in which neural remodelling occurs. As disease progresses from healthy state to pre-diseased state, abnormal increases in intracellular Ca^{2+} linked to increased neurotransmission can be detected in sympathetic neurones[279, 285]. This

cellular phenotype occurs before the overt clinical signs of hypertension and heart failure, and probably contributes to the sympathetic hyper-responsiveness when the disease is clinically evident. In parallel, parasympathetic impairment is also an early hallmark of autonomic impairment that further facilitates sympathetic dysfunction[111-112, 284]. Together, dysautonomia associated with both branches of the autonomic nervous system provide a trigger for abnormal conduction and rhythm disturbance[112,120]. Modified from Li & Paterson[272] and Zhang et al[295] **b** | Schematic showing the site of left cardiac sympathetic denervation. **c** | Implantable cardioverter defibrillator (ICD) shock free survival is better after bilateral cardiac sympathetic denervation (CSD) than after left CSD in patients with refractory ventricular tachycardia (VT) and structural heart disease. From Vaseghi et al[173].

Figure 6 | **Transcriptomics for the discovery of novel neuronal targets** Example of a study using RNA sequencing of stellate ganglia from normal rats and from rats with sympathetic hyperactivity, with validation in human samples and proposed disease model and therapeutic targets **a** | Gene ontology (GO) analysis of the differentially expressed genes showed that the top two over-represented 'molecular function' GO groups included 'extracellular ligand-gated ion channel activity' and 'phosphoric ester hydrolase activity'. Venn diagrams show upregulated (green) and downregulated genes (blue) in each GO group. Heat-map analysis of the RNA sequencing data showing the expression levels of differentially expressed genes, where green and blue represent upregulated and downregulated genes, respectively. **b** | The differential regulation of genes in the GO group of 'extracellular ligand-gated ion channel activity' was then validated with quantitative reverse transcription PCR in stellate ganglia from normal rats and rats with sympathetic hyperactivity, and in human samples of stellate ganglia. **c** | Kyoto Encyclopedia of Genes and Genome (KEGG) enrichment pathway analysis was used to identify relevant intracellular signalling pathways associated with the observed transcriptomic changes in rat stellate ganglia, revealing three KEGG pathways that were relevant to post-synaptic postganglionic signalling: 'circadian entrainment', 'dopaminergic synapse' and 'glutamatergic synapse'. The Venn diagram (left panel) represents the upregulated (green) and downregulated (blue) genes and the overlap between each of the KEGG groups. Finally, the KEGG signalling pathways were integrated in a cell model (right panel) highlighting the possible involvement of the identified transcriptomic changes on signalling and the link to abnormal regulation of intracellular Ca^{2+} levels and neurotransmission. For full methods see the original publication by Bradsley et al.[270]. Modified from Bardsley et al[270] with permission of authors.

Figure 7 | **Neurotransmitter switching in sympathetic neurons in prehypertension**. **a** | In healthy postganglionic sympathetic neurons , Ca^{2+}-dependent exocytosis facilitates the release of noradrenaline (NA), which activates β_1-adrenergic receptors (β_1AR) and β_2AR in cardiomyocytes. The increase in extracellular NA also activates presynaptic α_2-adrenergic receptors (α_2AR), which leads to a reduction in adenylyl cyclase activity in postganglionic sympathetic neurons through activation of inhibitory $G\alpha_i$ proteins.

Acute regulation of cAMP levels is mediated by cyclic nucleotide phosphodiesterases (PDEs). cAMP-dependent protein kinase A (PKA) activity increases the levels of intracellular Ca^{2+} through phosphorylation of the N-type Ca^{2+} channel (I_{CaN}) and the regulation of endoplasmic reticulum Ca^{2+} stores and mitochondrial Ca^{2+} release. **b** | In prehypertensive conditions, Ca^{2+}-dependent exocytosis in postganglionic sympathetic neurons facilitates the release of NA, neuropeptide Y (NPY) and adrenaline (Adr), which activate presynaptic β_1AR and β_2AR leading to higher cAMP generation, PKA activity and intracellular Ca^{2+} levels than in healthy neurons, thereby facilitating neurotransmission to the cardiomyocytes in a potentiating manner. This positive feedback occurs preferentially via β_2AR activation. Catecholamines can also come from the blood. Modified from Bardsley et al[40] with permission.

Figure 9 | **Potential mechanisms of cardiac sympatho-vagal transmission regulation**. a |Schematic representation of potential signal transduction pathways mediated by natriuretic peptides and nitric oxide (NO)–CAPON (also known as NOS1AP) in depolarized cardiac stellate neurons that target cGMP. Increased levels of CAPON in stellate neurons decreases neurotransmission and postsynaptic excitability by causing cGMP-dependent modulation of intracellular free Ca^{2+} concentration in stellate neurons. In cardiomyocytes, increased levels of CAPON decrease activation of the L-type Ca^{2+} current (I_{CaL}) and increased delayed rectifier K^+ current (I_{Kr}) via a NO-dependent pathway that shortens the action potential duration. Targeting pre-synaptic and postsynaptic sites with CAPON gene therapy might have therapeutic utility in patients with certain mutations associated with long QT syndrome. **b** | Representative action potential recordings in a CAPON-overexpressing cardiomyocyte showing that administration of the NO synthase inhibitor L-arginine methyl ester (L-NAME) reversal of action potential duration abbreviation. Modified from Li & Paterson[272] and Chang et al[292].

Glossary terms

Stellate ganglia

Refractory period

Electrical alternans

Cholinergic neurons

Optogenetic

VT storm

Evoked potentials

CLARITY