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Translational neurocardiology: preclinical models and cardioneural integrative aspects

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Abstract Neuronal elements distributed throughout the cardiac nervous system, from the level of the insular cortex to the intrinsic cardiac nervous system, are in constant communication with one another to ensure that cardiac output matches the dynamic process of regional blood flow demand. Neural elements in their various 'levels' become differentially recruited in the transduction of sensory inputs arising from the heart, major vessels, other visceral organs and somatic structures to optimize neuronal coordination of regional cardiac function. This White Paper will review the relevant aspects of the structural and functional organization for autonomic control of the heart in normal conditions, how these systems remodel/adapt during cardiac disease, and finally how such knowledge can be leveraged in the evolving realm of autonomic regulation therapy for cardiac therapeutics.

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Abbreviations AF, atrial fibrillation; ART, autonomic regulatory therapy; CHF, congestive heart failure; DRG, dorsal root ganglia; HF, heart failure; LV, left ventricle; MI, myocardial infarction; NTS, nucleus of the solitary tract; SCS, spinal cord stimulation; TRPV1, transient receptor potential vanilloid 1; VNS, vagus nerve stimulation.

Structural and functional organization of the cardiac nervous system: afferent signalling

Cardiac afferent neurons. Cardiac afferent neurons have been classified as being (i) mechanosensory, (ii) chemosensory or (iii) multimodal (transducing both modalities) in nature (Thoren *et al.* 1976; Thoren, 1977; Brown, 1979; Malliani & Lombardi, 1982; Foreman, 1999; Thompson *et al.* 2000; Kember *et al.* 2001; Armour, 2004; Fu & Longhurst, 2009). Afferent neuronal somata associated with sensory neurites in atrial, ventricular and intrathoracic major intravascular tissues are located in nodose and dorsal root ganglia (DRG) (Vance & Bowker, 1983; Armour *et al.* 1994; Hoover *et al.* 2008), as well as in intrathoracic extracardiac (Armour, 1983, 1986*a*) and intrinsic cardiac (Ardell *et al.* 1991; Beaumont *et al.* 2013) ganglia (Figure 1). Mechanosensory neurons are also associated with neurites embedded in the carotid

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sinus and thoracic aorta (Kirchheim, 1976; Zucker & Gilmore, 1991; Chapleau *et al.* 2001; Andresen *et al.* 2004). The sensory neurites associated with these somata transduce their local mechanical and/or chemical milieu in a differential manner, depending on the cardiac region transduced and the location of the ganglion in which their somata reside (Armour & Kember, 2004).

In the heart, a dense network of both myelinated and non-myelinated sensory fibres project to somata in the DRG of the thoracic spinal cord. These endings respond to many substances including hydrogen ions (Uchida & Murao, 1975), potassium (Uchida & Murao, 1975), bradykinin (Uchida & Murao, 1974), oxygen radicals (Ustinova & Schultz, 1994*a*; Huang *et al.* 1995), adenosine (Arora & Armour, 2003), ATP (Katchanov *et al.* 1996) and arachidonic acid metabolites (Staszewska-Barczak, 1983; Nerdrum *et al.* 1986; Sun *et al.* 2001; Fu *et al.* 2008). While single fibre recordings from afferents entering the thoracic DRG suggest that most of these endings are chemically sensitive,they may also respond to mechanical deformation, especially during intense ventricular contraction (Malliani *et al.* 1983). Studies by Zipes and coworkers (Inoue *et al.* 1988; Ito & Zipes, 1994) have demonstrated that one can interrupt

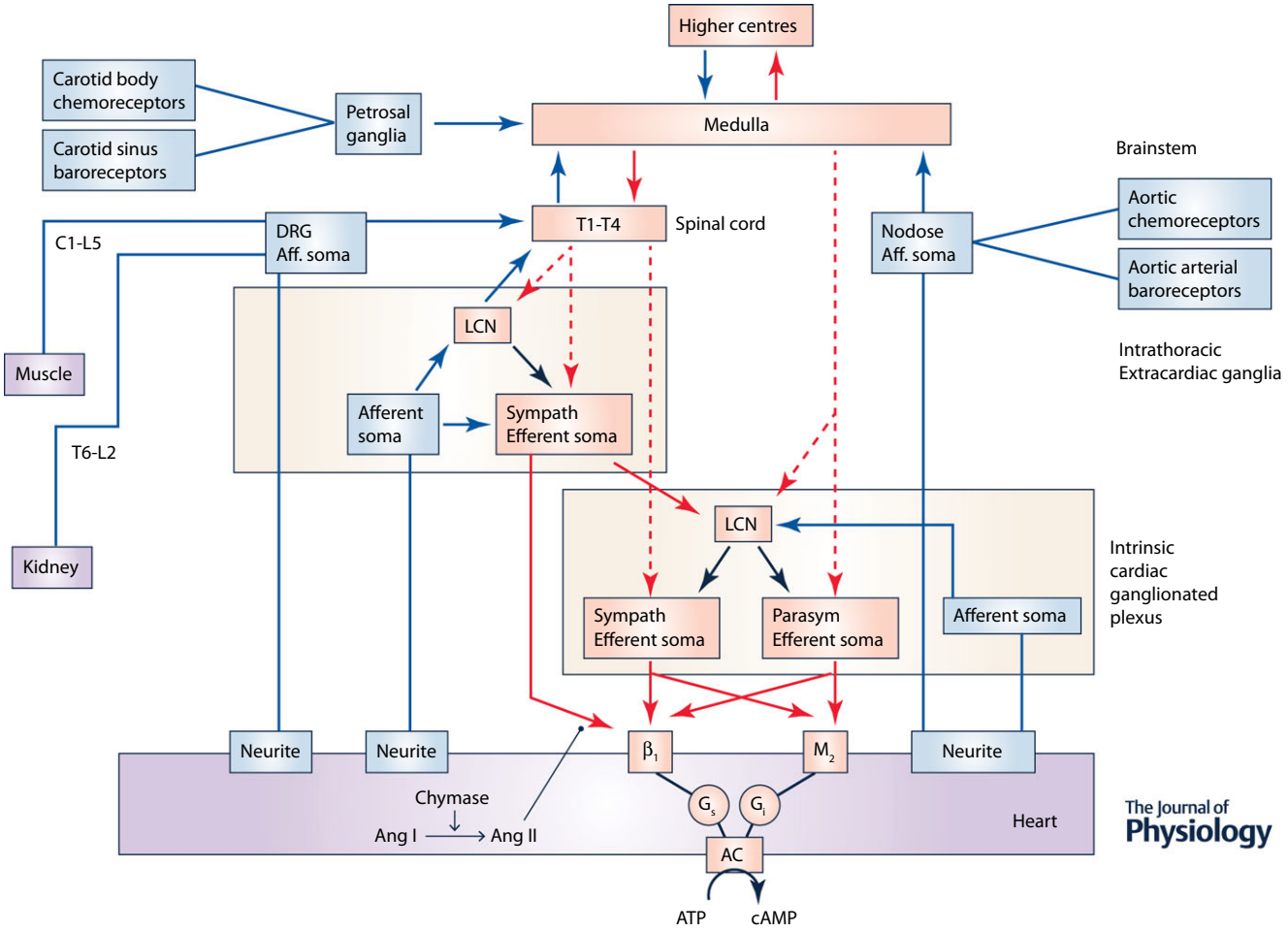

Figure 1. Network interactions occurring within and between peripheral ganglia and the central nervous system for control of the heart
The cardiac nervous system is composed of multiple (distributed) processing centres from which independent and interdependent neural feedback and feed-forward neural circuits interact to control regional cardiac electrical and mechanical function. Afferent projections are indicated in blue and efferent projections in red (dashed lines, preganglionic; continuous lines, postganglionic). The intrinsic cardiac nervous system (ICNS) possesses sympathetic (Sympath) and parasympathetic (Parasym) efferent postganglionic neurons, local circuit neurons (LCN) and afferent neurons. Extracardiac intrathoracic ganglia contain afferent neurons, LCN and sympathetic efferent postganglionic neurons. Neurons in intrinsic cardiac and extracardiac networks form nested feedback loops that act in concert with CNS feedback loops (spinal cord, brainstem, hypothalamus and forebrain) to coordinate cardiac function on a beat to beat basis. These systems demonstrate plasticity which underlies adaptations to acute and chronic stressors. Ang I, angiotensin I; Ang II, angiotensin II; AC, adenylate cyclase.

transmission via these fibres to abrogate reflex responses to bradykinin and nicotine by painting solutions of phenol on the surface of the heart, especially around the atrioventricular groove. Although the detailed distribution of these endings has not been elucidated, studies by Zipes, along with Zucker suggest that at least a large proportion of these fibres run near the surface of the left ventricle (Brandle *et al.* 1994; Zucker *et al.* 1995*a*,*b*). The use of new techniques such as CLARITY (Chung & Deisseroth, 2013) may help in the composition of a comprehensive cardiac map of the distribution and anatomical relationships of these nerve endings to other structures in the myocardium. CLARITY is a method for making tissue transparent using acrylamide-based hydrogels (Chung & Deisseroth, 2013). When used in conjunction with antibody labelling, CLARITY allows for highly detailed characterization of 3-D organization of heart and its innervation.

While some cardiac afferent somata reside in nodose and thoracic DRG, others are located in intrathoracic extracardiac and intrinsic cardiac ganglia (Armour & Ardell, 1994, 2004; Armour, 2008) suggesting a potential role for peripheral cardiocentric modulation of efferent autonomic neurons by local circuit neurons therein. Because their cardiac sensory endings transduce a variety of chemicals, including various neuropeptides (e.g. substance P, bradykinin and calcitonin gene related peptide), such primary afferents can also participate in 'axon reflexes' that initiate local inflammatory, vascular and permeability changes in the heart (Franco-Cereceda *et al.* 1993; White *et al.* 1993; Yaoita *et al.* 1994). While chronic activation of such sensory endings in cardiovascular disease states may not necessarily evoke symptoms (Foreman, 1999; Foreman *et al.* 2004), they may be important in initiating cardiac remodelling via a pro-inflammatory mechanism (Wang *et al.* 2014; Jänig, 2014*a*).

Glutamatergic transmission communicates information from the primary DRG sensory fibres arising in the heart to the dorsal horn of the thoracic spinal neurons (Foreman, 1999; Oliveira *et al.* 2003; Armour & Kember, 2004). This signalling can be importantly supplemented by the release of substance P, especially during cardiac stress (Hua *et al.* 2004; Ding *et al.* 2008*a*). Experimental studies have shown that occlusion of the coronary artery increased the release of substance P located in the superficial dorsal laminae I and II and deeper laminae including laminae III–VII, a change that was sustained for the duration of the occlusion (Hua *et al.* 2004). Furthermore, increased release of substance P during coronary artery occlusion was eliminated after bilaterally transecting the T2–T5 dorsal roots. Furthermore, molecular and morphological profiles have shown that substance P and preprotachykinin mRNA were upregulated in the T1–T5 dorsal horn during occlusion of the coronary artery (Guo *et al.* 2007), an effect that in part requires the involvement of transient receptor potential vanilloid 1 (TRPV1) receptors (Steagall *et al.* 2012). Thus, these data provide evidence that substance P released in the spinal cord dorsal horn is a key neuropeptide mediator that corresponds with the increased transmission of nociceptive information that results from myocardial ischaemia and is partially dependent on a TRPV1 mechanism.

The central pathways by which cardiac sympathetic afferent neurons participate in both pain and sympathetic responses are probably more complex than is appreciated currently. However, we do know that they ascend, in part, via the dorsal columns, spinothalamic and spinoreticular tracts to cortical terminations (Fig. 2) (Foreman, 1991, 1999). Along this path they project to mid- and hindbrain autonomic integrative areas. Recent studies have shown that cardiac afferent neurons influence neuronal activity in the paraventricular nucleus (Reddy *et al.* 2005; Affleck *et al.* 2012; Xu *et al.* 2013) and nucleus of the solitary tract (NTS) (Wang *et al.* 2006, 2007). These neuronal collections are ideally suited to modulate multiple cardiovascular reflexes, such as the arterial baroreflex and the chemoreflex (Reddy *et al.* 2005; Gao *et al.* 2007; Wang *et al.* 2007; Chen *et al.* 2015). As depicted below, activation of these endings during coronary ischaemia probably initiates cardiac sympathetic efferent reflexes that evoke lethal arrhythmias (Fukuda *et al.* 2015), an adverse response that can be mitigated by thoracic dorsal rhizotomy (Schwartz *et al.* 1976) or stellectomy (Bourke *et al.* 2010; Vaseghi *et al.* 2014). Activation of these cardiac afferents likewise is central to triggering neural remodelling associated with heart failure (HF) (Zucker *et al.* 2012; Wang *et al.* 2014). Neural remodelling is defined herein as induced changes in neuronal morphology, interconnectivity, phenotype and/or plasticity indicative of changes in active and/or passive membrane electrical properties on soma, dendrites and synapses.

Arterial baroreceptors. The arterial baroreflex is fundamental to dynamic regulation of blood pressure, both in response to everyday environmental stressors and for overall homeostasis (Kirchheim, 1976; Jänig, 2006; Sleight, 2014). Mechanosensory neurites, embedded in the outer walls of the aorta and carotid arteries, transduce local wall stretch. Their grouped firing yields activity patterns that sensitively track local arterial wall dynamics as reflective of arterial pressure waves (Kirchheim, 1976; Chapleau *et al.* 1995; Andresen *et al.* 2004). The larger myelinated fibres preferentially transduce dynamic changes in blood pressure; the small myelinated and C-fibres provide critical input on baseline levels of pressure (Seagard *et al.* 1990). This baroreceptor information is carried to the brainstem by central projections to evoke integrated reflex responses described below (see central reflexes).

Arterial chemoreceptors. Arterial chemoreflexes are subserved by peripheral chemosensory endings located in the carotid and aortic bodies, along with central chemoreceptors located in the medulla (Kumar & Prabhakar, 2012; Guyenet, 2014; Schultz *et al.* 2015*b*). The peripheral chemoreceptors preferentially respond to hypoxaemia; central chemoreceptors respond primarily to hypercapnia, a distinction that is not absolute (Kumar & Prabhakar, 2012; Guyenet, 2014). The sensitivity of these chemoreceptors can be modulated by a wide array of neurally derived and circulating substances; however, to date the exact molecular mechanisms of activation remain unclear (Kumar & Prabhakar, 2012; Guyenet, 2014).

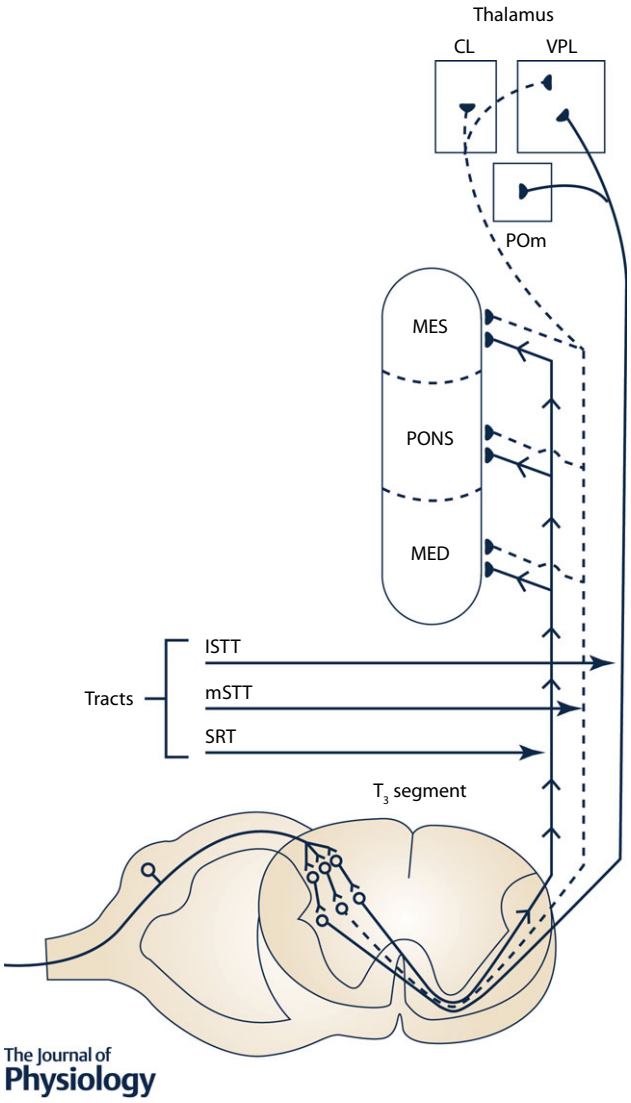

Figure 2. A schematic diagram of cardiac sympathetic afferents (T1–T6) transmitting nociceptive information in response to myocardial ischaemia that is relayed to ascending pathways terminating in supraspinal nuclei that participate in the signalling of cardiac pain
The spinoreticular tract (SRT), lateral spinothalamic tract (lSTT) and medial spinothalamic tract (mSTT) ascend to the reticular formation (medulla (MED), PONS, mesencephalon (MES)) and the ventral posterior lateral (VPL), central lateral (CL) and medial posterior (POm) nuclei of the thalamus. The cardiac sympathetic afferents also mediate sympatho-excitatory responses when stimulated by a variety of substances. Adapted from Foreman (1991) with permission.

These chemoreflexes, in addition to playing a significant role in the control of alveolar ventilation for CO_2 and O_2 homeostasis, also contribute to cardiovascular control (Kumar & Prabhakar, 2012; Guyenet, 2014). For instance, chemoreflex activation by hypoxia or hypercapnia increases blood pressure due to sympathetic efferent neuronal activation of the vascular beds (Kumar & Prabhakar, 2012; Guyenet, 2014). Reflex hyperventilation also increases central neuronal inputs from thoracic wall stretch receptor afferents. These act in a negative feedback manner to blunt the sympathetic activation discussed above (Somers *et al.* 1989*a*). There is also additional negative feedback on sympathetic outflow from arterial baroreceptors which are activated by chemoreflex-mediated increases in blood pressure (Somers *et al.* 1991). Such negative feedback mechanisms become more prominent in responses mediated by peripheral compared to central chemoreceptors (Somers *et al.* 1989*b*). Thus, chemoreceptor effects on the heart are influenced by cardiopulmonary and arterial baroreflex feedback. Conversely, there is the potential for feed-forward reflex control of peripheral chemoreflex function by sympathetic efferent neurons responding to peripheral chemoreception. As such, surgical removal of the right stellate ganglion reduces the reflex responses elicited by aortic chemoreceptors to hypoxia and hypotension (Anand, 1996). This excitatory effect of sympathetic nerves on peripheral chemoreceptors is thought to be mediated by ischaemic hypoxia secondary to vasoconstriction and reduction in blood flow to the chemoreceptor glomus (Anand, 1996).

Chronotropic responses to chemoreflex activation are more variable than the vasculature sympathetic responses when compared among species (Marshall, 1994). In humans, hypercapnia and hypoxia elicit a reflex increase in sympathetic efferent neuronal activity with a corresponding decrease in parasympathetic efferent neuronal inputs; a tachycardia thus accompanies the hyperventilation (Kara *et al.* 2003). However, during periods of apnoea, peripheral chemoreflex activation with hypoxia elicits a bradycardia (Kara *et al.* 2003). Such chemoreflex-mediated bradycardia, and corresponding peripheral sympathetically mediated vasoconstriction, is thought to contribute to the 'diving' reflex prominent in seals and evident in many mammals including primates (Marshall, 1994; Kara *et al.* 2003). These responses reduce myocardial energy demands, while maintaining normal arterial pressure in the face of the peripheral vasodilatory effects of hypoxia elicited by the periods of apnoea.

In humans, in pathophysiological states, when apnoea becomes frequent and recurring, these responses are detrimental, resulting in sustained sympathetic efferent neuronal activation to the periphery and autonomic imbalance to the heart (Fletcher, 2001).

Renal afferent neurons. Renal sensory neurons are subclassified as mechanosensitive or chemosensitive (Booth *et al.* 2015). Their mechanoreceptors are localized primarily to the renal parenchyma and wall of the renal pelvis (Niijima, 1975). Stimulation of these receptors evokes a centrally mediated reno-renal reflex (Ueda *et al.* 1967; Francisco *et al.* 1980; Kopp *et al.* 1985). Renal chemoreceptors (R1 and R2 types) are activated by the chemical environment in the intrarenal tissue and pelvis (Recordati *et al.* 1978, 1980). Stimulation of these receptors likewise evokes central-mediated sympatho-excitation (Recordati *et al.* 1982; Rogenes, 1982). Renal afferent neurons are primarily associated with unmyelinated C-fibres plus a much smaller population of Aδ-fibres (Knuepfer & Schramm, 1987). Their afferent inputs, projecting centrally via dorsal root ganglia (Donovan *et al.* 1983; Knuepfer & Schramm, 1987), can reflexly modulate sympathetic efferent neuronal outflows via spinal, medullary and higher centres (Calaresu & Ciriello, 1981; Wyss & Donovan, 1984; Xu *et al.* 2015). Thus, ablation of renal afferent inputs has the potential to alter autonomic outflows, both in the setting of hypertension (Esler *et al.* 2010; Bakris *et al.* 2015; Blankestijn *et al.* 2015) and HF (Booth *et al.* 2015).

Muscle afferent neurons. Skeletal muscle afferent neurons transduce the mechanical and chemical milieu of the musculature (Kaufman & Rybicki, 1987). Mechanical events in the musculature are transduced by mechanoreceptors associated with thinly myelinated group III fibres (Kaufman & Rybicki, 1987). On the other hand, local metabolic by-products are transduced by afferent neurons with unmyelinated (group IV) axons (Kaufman & Rybicki, 1987). Because these muscle receptors display polymodal transduction (Kaufman & Rybicki, 1987), exercise induced sensory activation can elicit substantial changes in autonomic neuronal cardiorespiratory adjustments, even during relatively mild exercise (Amann *et al.* 2011; Dempsey *et al.* 2014). Signals from muscle afferents integrate at multiple nexus points along the cardiac neuraxis and in humans can influence the activity of key autonomic midbrain nuclei such as the periaqueductal grey (Basnayake *et al.* 2011). Such sensory reflex activation preferentially increases sympathetic drive to the heart compared to the peripheral vasculature such that pressor responses are primarily due to increasing cardiac efferent neuronal output concomitant with central blood volume mobilization (Wyss *et al.* 1983; Sheriff *et al.* 1998; Crisafulli *et al.* 2003; Sala-Mercado *et al.* 2006).

Structural/functional organization of cardiac nervous system: cardiac motor neurons

Cardiac myocytes and coronary vessels are ultimately modulated by sympathetic and parasympathetic motor neurons (Randall *et al.* 1972; Randall, 1994), along with circulating hormones (Dell'Italia, 2011; Zucker *et al.* 2012). Efferent neuronal outflows from the central autonomic nervous system to the heart depend on both central (preganglionic) and peripheral neuronal mediated (postganglionic) reflexes (Zucker & Gilmore, 1991; Randall, 1994; Armour & Ardell, 2004; Coote, 2013). Within each nexus point of the neuronal hierarchy for cardiac control, from the central nervous system (CNS) to intrinsic cardiac ganglia (see Fig. 1), network interactions occurring within and between its levels are fundamental to network output control of cardiac motor neurons (Ardell, 2004; Armour, 2008; Herring & Paterson, 2009). The concept that the two efferent limbs of the cardiac nervous system function in a 'reciprocal' fashion wholly under central neuronal command (Levy, 1971; Levy & Martin, 1979; Dampney *et al.* 2002; Billman, 2006; Williamson *et al.* 2006) has been revised since it is now known that: (i) neurons in either of these motor limbs can be activated or suppressed concurrently (Armour, 2004; Coote, 2013); (ii) cardiocentric control exists within the thorax (Armour, 1983, 2008; Ardell *et al.* 1991) such that (iii) intrathoracic reflexes act to maintain bidirectional control of regional cardiac indices that is independent of the central nervous system (Armour *et al.* 1998; Schwartz, 2014; Vaseghi *et al.* 2014). That being said, a major scientific challenge remains to understand the essential elements of these interactions in determining not only the response to acute stressors, but also in the long-term adaptations associated with ageing and progressive cardiac disease.

Cardiac sympathetic efferent neurons. The sympathetic circuit features a core of pre-sympathetic efferent neurons in the brainstem that project to cardiac sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord (Guyenet, 2006; Guyenet *et al.* 2013). In humans, there is now functional evidence that mid-brain circuits also contribute to the modulation of sympathetic outflow. In particular, stimulation of the subthalamic (Thornton *et al.* 2002) and the periaqueductal grey (Green *et al.* 2005; Sverrisdottir *et al.* 2014) can drive sympathetic outflow. These caudal cervical and cranial thoracic spinal cardiac sympathetic efferent preganglionic neurons, in turn, project axons to sympathetic efferent postganglionic neurons in ganglia located in the neck and thorax (Norris *et al.* 1974, 1977; Buckley *et al.* 2016). While there is a degree of laterality in postganglionic projections arising from extracardiac sympathetic ganglia to the heart, there are substantial inputs to all chambers arising from somata in each major peripheral ganglion (Ardell *et al.* 1988;

Vaseghi *et al.* 2012; Ajijola *et al.* 2013). In addition, sympathetic efferent postganglionic neurons localized in each major intrinsic cardiac ganglionated plexus exert control over electrical and mechanical indices throughout the atria and ventricles (Butler *et al.* 1990*a*,*b*; Cardinal *et al.* 2009). That functional anatomy preserves cardiac control even when one or more intrinsic cardiac ganglionated plexuses are compromised (McGuirt *et al.* 1997; Randall *et al.* 1998, 2003; Leiria *et al.* 2011).

Cardiac parasympathetic efferent neurons. Cardiac parasympathetic efferent preganglionic neurons within the medulla oblongata (e.g. primarily nucleus ambiguus) (Hopkins & Armour, 1982; Massari *et al.* 1995; Dergacheva *et al.* 2014) target efferent postganglionic neurons distributed throughout the atrial and ventricular ganglionated plexuses. In turn, neurons in each of these distributed intrinsic cardiac ganglia exert control over electrical and mechanical function throughout both atria and ventricles (Yuan *et al.* 1994; Arora *et al.* 2003*b*; Cardinal *et al.* 2009). Such divergence of control also includes, in part, the bilateral nature of vagal preganglionic neuronal projections to all major atrial or ventricular ganglionated plexuses (Ardell & Randall, 1986; McGuirt *et al.* 1997; Yamakawa *et al.* 2014). Taken together, these data support the thesis that the intrinsic cardiac nervous system, as a collective, acts to coordinate regional cardiac electrical and mechanical function (Armour, 2008; Kember *et al.* 2011), even when operating chronically disconnected from higher centres (Ardell *et al.* 1991; Murphy *et al.* 2000; Vaseghi *et al.* 2009).

Local circuit neurons. Local circuit neurons are located in all intrathoracic ganglia, including those distributed on the heart. They subserve interactive processing of information by neurons in and among peripheral autonomic ganglia (Ardell, 2004; Armour, 2004, 2008). The cardiac and intrathoracic vascular mechano- and chemosensory inputs discussed above are processed within intrinsic cardiac and intrathoracic extracardiac ganglia primarily by these local circuit neurons (Armour, 1983, 1994; Ardell *et al.* 1991; Murphy *et al.* 2000). They also transduce inputs from the central nervous system (Armour, 1985, 1986*a*,*b*; Beaumont *et al.* 2013). As such, peripheral interactive local circuit neurons are fundamental to coordinating motor neuronal outputs to the heart (Armour & Ardell, 2004; Armour, 2008).

Structural/functional organization of cardiac nervous system: reflex control

Peripheral intrinsic cardiac reflexes. The entire cardiac neuronal hierarchy acts as a distributive processor, employing multiple nested feedback control loops to modulate cardiac function throughout each cardiac cycle that together initiate both fast-acting, short-loop as well as longer-latency reflex control over regional cardiac indices (Armour, 2008). In such a scenario, intrinsic cardiac neurons are ultimately under the control of more centrally located intrathoracic extracardiac neurons along with the central nervous system (Ardell, 2004). The population of intrinsic cardiac neurons that receive obligatory pre- to postganglionic efferent inputs is less than 15% of the total population (Gagliardi *et al.* 1988; Armour & Hopkins, 1990*a*,*b*; Beaumont *et al.* 2013). A substantial population of the local circuit neurons responds to both sympathetic and/or parasympathetic inputs (Beaumont *et al.* 2013; Rajendran *et al.* 2016). Being involved in inter-ganglionic interactions, the local circuit neurons subserve critical roles in integrated control of cardiac function among peripheral ganglia and between peripheral and central components of the cardiac nervous system. These local circuit neurons may serve as the primary targets for pharmacological and/or bioelectric therapeutic strategies to mitigate the hyperdynamic cardiac reflex responses accompanying progression of cardiac disease (Armour, 2008; Gibbons *et al.* 2012; Beaumont *et al.* 2015; Hardwick *et al.* 2015). The intrinsic cardiac nervous system is the cornerstone of cardiocentric reflexes; that is, reflexes that are confined to the periphery and with the heart as their target organ.

Peripheral intrathoracic, extracardiac reflexes. Cardiocentric reflexes are also dependent upon neuronal somata in superior cervical and intrathoracic (superior and middle cervical, stellate and mediastinal) sympathetic ganglia, forming intrathoracic, extracardiac reflexes that control intrinsic cardiac motor and local circuit neurons (Armour & Ardell, 2004; Armour, 2010). Intrathoracic extracardiac reflexes receive both excitatory and inhibitory inputs from spinal cord neurons that, in particular, influence the regulation of regional cardiac electrical and mechanical function (Armour, 1985, 1986*a*,*b*; Ardell *et al.* 2009).

Peripheral somato-autonomic reflexes. Muscle sensory neurites transduce changes in skeletal mechanical events, along with the concentration of metabolic by-products (Kaufman & Rybicki, 1987). Activation of these afferents can change spinal driven efferent autonomic outputs, and thereby contribute to the cardiac adjustments elicited by dynamic exercise (Amann *et al.* 2011; Dempsey *et al.* 2014). Normally, during exercise, activation of these afferents preferentially increases sympathetic efferent neuronal inputs to the heart, thereby mediating a pressor response associated with increasing cardiac output (Sheriff *et al.* 1998; Crisafulli *et al.* 2003; Sala-Mercado *et al.* 2006). Increase in ventricular function occurs despite restraint involving metabolite induced coronary arterial vasodilatation that can overcome increased sympathetic

drive that targets coronary arterial α-adrenergic receptors (Coutsos *et al.* 2010). These changes involve at least three different control mechanisms: (i) feed-forward effects of central command, (ii) feedback initiated by the exercise-induced pressor reflex (secondary to activation of skeletal muscle afferents), and (iii) feedback effects mediated by arterial and cardiopulmonary baroreflexes (Rowell *et al.* 1996).

Central integration. Tonic activity of autonomic neurons reflects the output of an integration process within central neuronal networks that set a base level of regulatory influence even in the unstressed, resting cardiovascular state. The sympathetic network contains a central sympathetic pattern generator with a high level of intrinsic, tonic activity (Guyenet, 2006; Jänig, 2014*b*). At rest, inhibitory mechanisms (GABA) activated by ongoing arterial baroreceptor afferent discharge restrains central sympathetic network activity and thus sympathetic output to the periphery (Schreihofer & Guyenet, 2002; Guyenet, 2006). In contrast, cardiac parasympathetic preganglionic neurons display lower levels of basal activity (McAllen & Spyer, 1978; Mendelowitz, 1999; McAllen *et al.* 2011). For cardiac directed parasympathetic preganglionic efferent neurons, these neurons have no intrinsic pattern generators but at rest are activated by ongoing excitatory inputs (Kunze, 1972; Mendelowitz, 1999). This resting primary afferent input to cardiac vagal preganglionic neurons arises primarily from arterial baroreceptors with lightly myelinated, A-type axons, since these have pressure thresholds somewhat below normal prevailing mean arterial pressure at rest (Andresen & Kunze, 1994; Andresen *et al.* 2012). Cardiac parasympathetic preganglionic efferent neuron activity activates subsets of parasympathetic efferent postganglionic neurons distributed throughout the intrinsic cardiac nervous system that ultimately act as conduits to cardiac myocytes such as those of the sinoatrial node to control heart rate and the rest of the heart (Edwards *et al.* 1995; McAllen *et al.* 2011; Beaumont *et al.* 2013; Hardwick *et al.* 2014).

Peripheral reflexes. Perhaps less recognized in such control is the impact of dual cranial and spinal paths of primary cardiovascular sensory afferent neurons (Armour & Kember, 2004; Kember *et al.* 2011, 2013*a*). With respect to overall control, the heart as well as the vascular system provide two basic sources of primary afferent inputs: (i) those directed to the brainstem at the NTS, and (ii) those projecting to spinal cord neurons (thus, cranial visceral afferent neurons such as in nodose *vs.* spinal dorsal root ganglia, respectively). The cranial nerve activated pathways, in most instances, suppress cardiac functions in a negative feedback fashion (Guyenet, 2006; Browning & Travagli, 2011; Guyenet *et al.* 2013). Conversely, spinal visceral afferent neurons can elicit deleterious positive feedback to sympathetic effector neurons – a form of positive feedback (Brown, 1979; Ardell *et al.* 1982; Malliani & Montano, 2002; Grundy, 2004). Activation of cardiac afferent inputs to NTS generally are not consciously perceived, whereas activation of cardiac spinal afferent inputs can activate perceptible pain along with indirect pathways that impact brainstem autonomic centres (Foreman, 1999).

Cardiac primary afferent axons are subclassified as belonging to two distinct cellular phenotypes that are indicated by differences in axon conduction velocity: (i) fast conducting myelinated A-fibres and (ii) slowly conducting unmyelinated C-fibres. The cardiovascular sensors have characteristically different sensitivities with A-fibre afferent axons associated with lower physiological thresholds and higher discharge rates compared to C-fibre afferent axons with more limited and often irregular discharge (Armour & Kember, 2004). C-class axons constitute the overwhelming majority of these cardiovascular primary afferents. For instance, 80–90% of arterial baroreceptor neurons are of the C-fibre phenotype (Andresen *et al.* 2012). C-fibre activation elicits strong cardiovascular reflexes at low frequencies of activation compared to A-fibre afferents which require higher frequencies for discernible reflex responses (Fan *et al.* 1999). While A- and C-fibre cardiovascular afferent neurons synaptically terminate in similar regions of the NTS, the afferent information generally does not mix to excite the same NTS neuron (Donoghue *et al.* 1981; Mifflin, 1996). As such, these neural circuits provide highly separate reflexes that are dependent upon either A-fibre or C-fibre afferent neural inputs to the medulla (Jin *et al.* 2004; Andresen & Peters, 2008; Peters *et al.* 2011). Many of these neurons receiving afferent inputs then directly send axons to project out of NTS to other destinations within the brainstem such as nucleus ambiguus (e.g. cardiac parasympathetic preganglionics) or beyond to areas such as the periventricular nucleus of the hypothalamus (Bailey *et al.* 2006). Some NTS neurons are only indirectly connected to the primary afferents and these higher order sensory neurons often also connect to different brainstem or forebrain areas including those in the ventromedial and raphe nuclei as well as key hypothalamic nuclei, regions which strongly impact autonomic efferent control (Dampney *et al.* 2005).

To date, the gains for parasympathetic and sympathetic baroreflex appear to be independent of one another and when they change with pathophysiology no direct correlation has been found, even though cranial baroreceptor afferents contribute to both (Rudas *et al.* 1999). Most measures of baroreflex gain, especially those used clinically or for *in vivo* chronic recordings, depend primarily on activating myelinated reflex pathways because only afferents with lower afferent transduction thresholds are engaged at normal pressures (Andresen

et al. 2012). Similar independence appears in chronic modification of autonomic pathways in congestive heart failure where exercise training improves parasympathetic, but not sympathetic, baroreflex control of rabbit (Liu *et al.* 2002) and human hearts (Sheldahl *et al.* 1994). The presence and release of neuropeptides onto their G-protein coupled receptors in central autonomic circuits may well be responsible for acute and chronic plasticity of the system (Stern *et al.* 2012). Beyond NTS, interconnections between anatomical sites show broad pairings of reciprocal connections; but little is known about their specific functions or even broad points such as A-fibre/C-fibre convergence. Only fragments of these relationships suggest potential impacts of these interactions between NTS and key brainstem or supramedullary regions, such as the paraventricular nucleus of the hypothalamus. Thus, growing evidence suggests that central pathways are likely to be much more discrete in organization than currently appreciated with specific contributions to the integrated whole in cardiovascular control.

Questions

Circuit map for normal cardiac control

- What are the critical neural elements (peripheral *vs.* central) for cardiac control that translate from animal models to the human condition?
- What are the critical inherent differences in cardiac neural control that predispose individuals to cardiac disease and, as such, what is/are the appropriate biomarker(s) to assess this potential?
- What are the effects of ageing on integrated neural control of the heart?

Neuraxial transduction of cardiac pathology

Overview. Cardiac disease involves maladaptive interactions that occur not only at the level of the cardiomyocyte, but also among both local and more remote neurons regulating cardiac function (Zipes *et al.* 2006; Houser *et al.* 2012; Park *et al.* 2012; Shinohara *et al.* 2012; Ajijola *et al.* 2015). In the presence of cardiac pathology the cardiac nervous system adapts in a reactive attempt to maintain adequate cardiac electrical and mechanical function (Armour, 2008; Fukuda *et al.* 2015). While cardiac hierarchical control readily reorganizes in response to normal physiological perturbations (Kember *et al.* 2011), it can be compromised as it responds to the demands of deteriorating cardiac function over longer time scales (e.g. heart failure) or sudden shifts in demand initiated by events such a myocardial ischaemia (Vaseghi & Shivkumar, 2008; Kember *et al.* 2013*b*; Florea & Cohn, 2014; Fukuda *et al.* 2015). This section deals primarily with adaptations in the neural processing associated with ischaemic and non-ischaemic heart disease, focusing on translating information derived from preclinical studies. Figure 3 schematically represents specific aspects of these adjustments, focusing on adaptation to myocardial infarction. The reader is referred to the companion White Papers on the neural–myocyte microenvironment (Habecker *et al.* 2016) and clinical manifestations (Shivkumar *et al.* 2016) for additional information. Across all three White Papers, the fundamental premise is that the progression of cardiac disease reflects the dynamic interplay between neurohumoral and cardiac end-effectors. Specifically, multiple factors are involved in disease progression including cardiac substrates, the neural–myocyte interface, myocyte energetics, vascular and interstitial hormonal systems, peripheral and central mediated reflexes of the cardiac nervous system, and the interplay with higher centre neurons.

Cardiac pathology can induce both short-term and long-term effects on neural networks involved in cardiac control, some giving rise to exaggerated reflex responses in some while others are blunted (Zucker *et al.* 2012; Schultz *et al.* 2013; Florea & Cohn, 2014; Fukuda *et al.* 2015). Such neuronal remodelling has the potential to develop conflicts between central and peripheral reflexes of the cardiac nervous system (Kember *et al.* 2013*b*), a condition predisposing to arrhythmia formation and/or deterioration of contractile function (Armour, 2008; Florea & Cohn, 2014; Fukuda *et al.* 2015). Central sensitization can occur because of plasticity of neurons within the spinal grey matter. Continual intense transmission of action potentials arising from a cardiac site of injury or inflammation, via nociceptive afferent neurons, can elicit cellular processes that increase the excitability of cell membranes, facilitate synaptic strength, and decrease inhibitory transmission within the nervous system (Latremoliere & Woolf, 2009). These cellular changes can enhance the responsiveness of spinal neurons and neurons that transmit nociceptive information to supraspinal levels with a resultant increase in pain sensation (Woolf & Salter, 2000).

The relevance of the cardiac neuronal hierarchy in myocardial ischaemia. Evidence exists indicating that central neuronal sensitization may be associated with changes in glial function during myocardial ischaemic episodes. Marked increases in the central neuronal release of glutamate, substance P and other neurotransmitters resulting from the bombardment of spinal afferent neurons may trigger enhanced excitation of glia in neuronal domains (Milligan & Watkins, 2009). In addition, numerous inflammatory mediators can be released from glia, including tumour necrosis factor α (TNF-α), which has been shown to be associated with myocardial ischaemia. In fact, occlusion of the rat left anterior descending coronary artery was found to

upregulate TNF-α in the dorsal horn 30 min after onset; such upregulation could be sustained for 6 h (Niu *et al.* 2009). Myocardial ischaemia engages multiple afferent pathways, impacting both peripheral and central reflex arcs (Ustinova & Schultz, 1994*b*; Schultz & Ustinova, 1996; Armour, 1999; Malliani & Montano, 2002). The cardiac nervous system exhibits short-term memory (Armour *et al.* 2002; Ardell *et al.* 2009) and longer-term plasticity (Kember *et al.* 2013*a*; Wang *et al.* 2014; Fukuda *et al.* 2015). Such neural influences represent a major determinant for cardiac control in the setting of progressive cardiovascular disease.

The relevance of the cardiac neuronal hierarchy to cardiac arrhythmia induction. It has been recognized for some time that autonomic neuronal dysfunction plays a significant role in the induction and maintenance of atrial or ventricular arrhythmias (Armour *et al.* 1972; Gelband *et al.* 1977; Scherlag *et al.* 2006; Chen *et al.* 2014; Fukuda *et al.* 2015; Zipes, 2015). Pathological stressors have the potential to disrupt nested feedback loops within the cardiac neural hierarchy, sometimes with lethal consequences (Schwartz, 1984, 2001; Chen *et al.* 2001; Billman, 2006; Scherlag & Po, 2006; Shen & Zipes, 2014; Ajijola *et al.* 2015; Fukuda *et al.* 2015; Gardner *et al.* 2015). In fact, derangement of neural processing throughout the cardiac neural hierarchy in response to transducing pathologies such as regional ventricular ischaemia frequently give rise to altered efferent neuronal outputs to different cardiac regions (Armour, 1999; Kember *et al.* 2013*b*). This includes alterations in reflex processing within intrinsic cardiac (Huang *et al.* 1993;

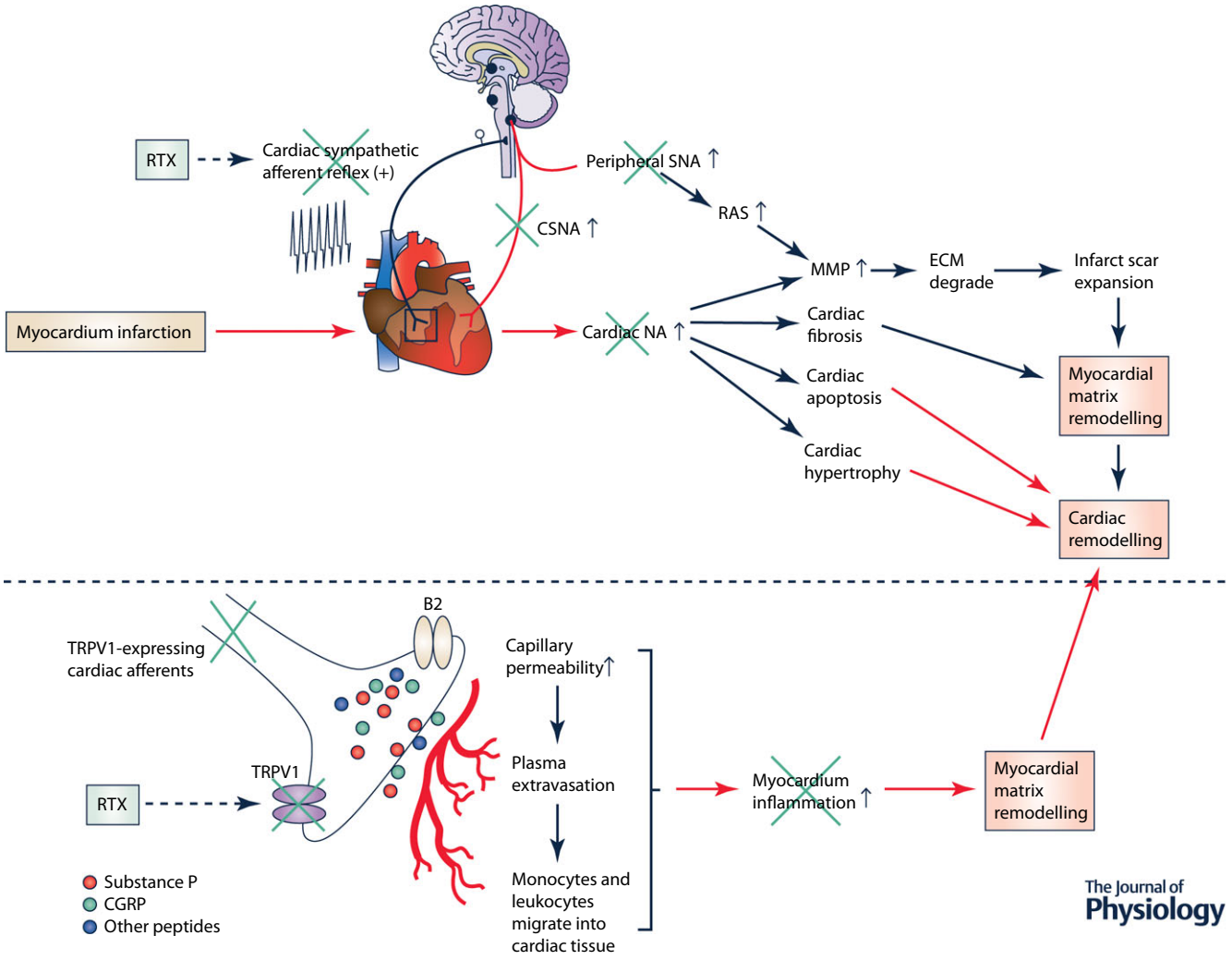

Figure 3. A schematic overview of a potential role for cardiac sympathetic afferents in mediating both sympatho-excitatory and cardiac remodelling effects in heart failure and the use of resiniferatoxin (RTX) to antagonize these effects by ablation of TRPV1-expressing afferents
SNA, sympathetic nerve activity; CSNA, cardiac sympathetic nerve activity; MMP, matrix metalloprotease; ECM, extracellular matrix; NA, noradrenaline. RAS, renin angiotensin system; CGRP, calcitonin gene-related peptide.

Foreman *et al.* 2000) and extracardiac intrathoracic ganglia (Armour *et al.* 1998; Ardell *et al.* 2009; Ajijola *et al.* 2015) as well with central (spinal and supraspinal) reflexes (Malliani & Montano, 2002; Ding *et al.* 2008*a*; Fu & Longhurst, 2009; Zucker *et al.* 2012; Wang *et al.* 2014).

Enhanced cardiac sympathetic drive promotes myocyte calcium influx and increases the inotropic state of the heart, which results in increased myocardial oxygen demand (Levy & Martin, 1979; Randall, 1994; Zucker *et al.* 2012). This can exacerbate the harmful effects of pre-existing cardiac ischaemia to precipitate life-threatening ventricular arrhythmias (Opie & Clusin, 1990; Lubbe *et al.* 1992). This is particularly prevalent when there is an abnormal cardiac structural phenotype to support re-entrant pathways, such as in ischaemic and dilated cardiomyopathies, hypertrophic cardiomyopathy, and arrhythmogenic right ventricular cardiomyopathy (Florea & Cohn, 2014; Fukuda *et al.* 2015). Excessive adrenergic activity is unsurprisingly a negative prognostic indicator post myocardial infarction (MI) (Kleiger *et al.* 1987; La Rovere *et al.* 1998), as well as during congestive cardiac failure (Cohn *et al.* 1984; Nolan *et al.* 1998). High sympathetic drive to the heart can also precipitate arrhythmia, particularly when there is an abnormal cardiac electrophysiological substrate such as in long QT syndrome (LQTS) types 1 and 2 (Shen & Zipes, 2014).

In randomized controlled trials β-blockers, introduced over 50 years ago, represent the only anti-arrhythmic pharmacological agent known to improve mortality post MI or in chronic congestive HF (ISIS-1, 1986; CIBIS-II, 1999). Moreover, improving cardiac cholinergic neurotransmission is notoriously difficult to achieve pharmacologically, although gene transfer of neuronal nitric oxide synthase (nNOS) into intracardiac ganglia can facilitate cholinergic transmission (Heaton *et al.* 2007). Thus, recently there has been a surge in interest of utilizing interventional procedures that are aimed at modulating cardiac sympathovagal balance. In fact, recent case series have advocated stellectomy (Ajijola *et al.* 2012; Schwartz, 2014), thoracic epidural anaesthesia (Bourke *et al.* 2010) or renal sympathetic nerve denervation (Bradfield *et al.* 2014), as well as vagal nerve stimulation (Huang *et al.* 2015) as potential approaches to various cardiac pathologies.

Under normal conditions the cardiac cholinergic signalling prevents intracellular calcium overload and slows heart rate. Its activation can raise the threshold for ventricular fibrillation induction (Ng *et al.* 2007) and this appears to be dependent on an nNOS, muscarinic receptor dependent pathway (Kalla *et al.* 2016). Conditions that promote high vagal tone such as exercise training (Danson & Paterson, 2003; Billman, 2006) protect against cardiac mortality (Cole *et al.* 1999). On the other hand, impaired vagal function is a negative prognostic indicator in congestive cardiac failure patients (La Rovere *et al.* 1998). It is important to note that in conditions associated with the *SCN5A* mutation causing LQT3 or Brugada's syndrome, vagal tone can paradoxically trigger ventricular arrhythmias; the mechanistic basis of this observation remains ill-understood (Shen & Zipes, 2014).

Cholinergic stimulation targeting cardiac myocyte muscarinic receptors counters sympathetic changes by inhibiting adrenergic and cyclic adenosine monophosphate (cAMP)-protein kinase A dependent increases in L-type calcium current I_{CaL} via direct and indirect pathways. Direct inhibition of β-adrenergic receptor function occurs via interactions of adenylate cyclase with the α subunit of pertussis toxin (PTX)-sensitive G_i/G_o proteins of the M_2 receptor (Sunahara *et al.* 1996). Indirect effects may also be dependent upon the generation of NO by endothelial nitric oxide synthase (eNOS), thereby leading to cGMP production and increased phosphodiesterase-2 activity that initiate breakdown of cAMP and subsequent reduction in I_{CaL} (Balligand *et al.* 1993; Han *et al.* 1994) – a controversial concept (Vandecasteele *et al.* 1999). The main functional role for NO appears to be presynaptic at the level of the cholinergic ganglia in modulating acetylcholine release (Herring & Paterson, 2001; Herring *et al.* 2002; Paton *et al.* 2002) (see accompanying White Paper Habecker *et al.* 2016 for additional details). These cellular mechanisms contribute to changes in ventricular electrical events, as evidenced by action potential duration (APD) prolongation and flattening of the electrical restitution curve – along with reduction in spatial heterogeneity of these variables (Ng *et al.* 2001). Recent evidence has also shown an increase in myocyte connexin-43 expression in response to chronic vagal nerve stimulation, a change that appears to promote homogeneity in conduction velocity (Ando *et al.* 2005).

Given the above, excessive activation of subpopulations of intrinsic cardiac neurons by co-activating their extracardiac cholinergic and adrenergic inputs is known to initiate cardiac arrhythmias (Schwartz *et al.* 1992; Chen *et al.* 2001; Armour *et al.* 2005; Billman, 2006; Lujan *et al.* 2010; Gibbons *et al.* 2012; Beaumont *et al.* 2013). In fact, this propensity appears to reside with excessive, stochastic interplay initiated among select neuronal elements within the intrinsic cardiac nervous system – specifically its local circuit neurons (Gibbons *et al.* 2012; Beaumont *et al.* 2013). Although our knowledge of the role that local circuit neurons play in intrinsic cardiac and intrathoracic extracardiac neuronal interactions remains limited, it appears that this population of neurons is responsible for most integrative control that occurs in the periphery (Armour *et al.* 1998; Waldmann *et al.* 2006; Armour, 2008; McAllen *et al.* 2011). As such, it has been suggested that targeting this population therapeutically might act to normalize information processing within the

intrathoracic nervous system to reduce excessive imbalance and thereby mitigate this arrhythmogenic substrate (Gibbons *et al.* 2012).

The cardiac neuronal hierarchy in heart failure. In the failing heart changes occur not only in the cardiac musculature, but also in the neurohumoral control system that modulates its musculature (Zipes *et al.* 2006; Armour, 2008; Vaseghi & Shivkumar, 2008; Mill *et al.* 2011; Zucker *et al.* 2012). With respect to the cardiac neuronal hierarchy, in HF remodelling can occur at multiple levels from the intrinsic cardiac nervous system (Bibevski & Dunlap, 1999, 2004, 2011; Arora *et al.* 2003*a*; Hardwick *et al.* 2008, 2009, 2014; Shinohara *et al.* 2012) to intrathoracic extracardiac neurons (Tallaj *et al.* 2003; Hankes *et al.* 2006; Han *et al.* 2012; Nguyen *et al.* 2012; Ajijola *et al.* 2015), extending up to central neural processing circuits associated with the arterial baroreflex (Zucker *et al.* 2012). Alterations in neurohumoral control also include the renin–angiotensin–aldosterone system (RAAS) and circulating catecholamines (Dell'Italia, 2011; Mill *et al.* 2011).

Altered intrathoracic reflex control in heart failure. Altered afferent neuronal feedback to intrathoracic (intrinsic cardiac and stellate/middle cervical ganglion) neurons in the transduction of myocardial infarction leads to remodelling of the intrathoracic cardiac nervous system, both intrinsic cardiac and extracardiac. The altered afferent input so induced transduces the (i) ventricular substrate subsequent to infarction (Crow *et al.* 2004; Glukhov *et al.* 2010, 2012; Belevych *et al.* 2012), (ii) local efferent neural sprouting (Cao *et al.* 2000; Zhou *et al.* 2004; Ewert *et al.* 2008; Gardner *et al.* 2015), (iii) stress-induced changes in the collagen matrix (Dobaczewski *et al.* 2010; Ulasova *et al.* 2011; Wei *et al.* 2012), and (iv) disruptions in the regional cardiac milieu as reflective of local contractility and associated metabolic changes (Mollema *et al.* 2007; Antoni *et al.* 2011). Taken together, these adaptations lead to afferent dependent disruptions in central and peripheral reflexes for cardiac control (Ahonen *et al.* 1975; Armour, 1999, 2008; Chen *et al.* 2001; Zucker *et al.* 2012; Florea & Cohn, 2014; Fukuda *et al.* 2015).

Altered baroreflex function in heart failure. In HF patients, haemodynamic, metabolic, humoral (e.g. renin–angiotensin system) and inflammatory response alterations are involved in initating excessive sympatho-excitation (Dell'Italia, 2011; Zucker *et al.* 2012; Florea & Cohn, 2014; Jänig, 2014*a*). A prolonged sympatho-excitatory state exacerbates HF (Zucker *et al.* 2012; Florea & Cohn, 2014). Reduced baroreflex control of heart rate, a dynamic baroreflex function, has been shown to correlate closely with severity and, as a consequence, poor prognosis in HF patients with reduced ejection fraction (HFrEF) (Florea & Cohn, 2014). Blunted baroreflex control of heart rate also occurs in HF patients with preserved ejection fraction (HFpEF) (Borlaug *et al.* 2006; Borlaug, 2014). In fact, compromised baroreflex control of cardiac function is fundamental to the evolution of HF (Zucker *et al.* 2012; Floras & Ponikowski, 2015).

One major component of altered baroreflex control in heart failure is induced changes in the end-organ innervation profile. The hyperdynamic sympatho-excitation (Zucker *et al.* 2012; Florea & Cohn, 2014; Fukuda *et al.* 2015), with resultant changes in end-organ receptor coupling (Lefkowitz, 2013), also includes alterations in regional sympathetic innervation. Specifically, there is progressive loss of end-terminus nerve terminals and their functional apparatus to release and re-uptake catecholamines (Himura *et al.* 1993). Preserving the integrity and function of sympathetic nerve terminals may be an important approach in the management of heart failure (Liang, 2003). The reader is referred to the companion White Paper by Habecker *et al.* for an expanded discussion on cellular and molecular adaptions to heart disease (Habecker *et al.* 2016).

In combination with influencing cardiac indices, the baroreflex exerts a substantial control over the peripheral vasculature (Kirchheim, 1976; Zucker & Gilmore, 1991). Funakoshi *et al.* (2014) examined the impact of baroreflex function on volume tolerance in rats. They demonstrated that rats maintained with constant carotid sinus pressure levels lost the ability to buffer elevation of left atrial pressure over time as well as arterial pressure changes in response to intravenous volume infusion. Sakamoto *et al.* (2016) demonstrated that sinoaortic denervation, which is an established animal model of impaired baroreflex, destabilizes left atrial pressure dynamics along with arterial pressure regulation in freely moving rats, thereby inducing recurrent episodes of high left atrial pressure. Additionally, they demonstrated that high salt intake exacerbates such volume intolerance (Sakamoto *et al.* 2016).

Sakamoto *et al.* (2015) reported that integrated baroreflex control of blood pressure depends in large part on vascular properties that contribute to altered baroreflex regulation of heart rate ($4 \pm 2\%$), arterial vascular resistance ($32 \pm 4\%$), end-systolic elastance ($14 \pm 4\%$) and stressed blood volume ($39 \pm 4\%$). Thus, it appears that the baroreflex markedly influences vascular capacitance to buffer blood pressure changes. These data indicate the dynamic nature that baroreflex function plays in blood volume shifts in normal and evolving pathological states. Given sufficient stress and time, such hyperdynamic compensatory responses transition to a decompensated state in end-stage pathology (Zucker *et al.* 2012; Florea & Cohn, 2014; Fukuda *et al.* 2015). Understanding the mechanistic basis for such adaptions as well

as their pathological outcomes provides fertile ground for evolving new targeted therapies to mitigate cardiac disease processes associated with altered baroceptor function.

Altered skeletal muscle reflex function in heart failure. Activation of skeletal muscle afferents in heart failure often causes marked increases in sympathetic activity and circulating levels of vasoactive hormones which elicits substantial peripheral vasoconstriction (Hammond *et al.* 2000; Smith *et al.* 2003; Crisafulli *et al.* 2007; Koba *et al.* 2008). Increased α-adrenergic coronary vasoconstriction contributes to the limited ability to raise ventricular function (Coutsos *et al.* 2013). This shift in the reflex pressor responses, from raising cardiac output to increased peripheral vasoconstriction, may involve depressed buffering by the arterial baroreflex in heart failure (Kim *et al.* 2005).

Altered chemoreflex function in heart failure. There is compelling evidence that cardiovascular chemoreflexes, particularly those involving peripheral chemoreflexes, are tonically activated in several disease states, including hypertension, renal failure, HF, diabetes and sleep apnoea, with detrimental effects on cardiorespiratory function (Hering *et al.* 2007; McBryde *et al.* 2013; Conde *et al.* 2014; Schultz *et al.* 2015*b*). Although the specific mechanisms responsible for enhancing chemoreflexes in these disease states are by no means fully defined, it appears likely that they differ based upon the aetiology of the disease. As such, to date there is no clear unifying molecular, cellular or integrative process delineating their response characteristics in such pathologies.

However, it is clear that the pathophysiological effects of tonic chemoreflex activation share many common features across various cardiac diseases. Given the above, current evidence indicates that it is likely that changes occur at multiple levels of reflex transduction. These include: (i) increased sensitivity of the sensory chemoreceptors to local tissue excitatory mediators; (ii) altered central integration of these increased chemoreceptor inputs; and (iii) altered integration of efferent sympathetic and parasympathetic neuronal outflows to the heart, vasculature, lungs and other organs. The hallmark of such pathological control is defined by increases in chemoreflex activity that can override negative feedback mechanisms such as arise as a consequence of cardiac, major vasculature and pulmonary milieu transduction with resultant hyperdynamic activation of sympathetic outflows to the heart and vasculature (Hering *et al.* 2007; Paton *et al.* 2013; Schultz *et al.* 2013; Conde *et al.* 2014). Such sympatho-excitation not only contributes to increased cardiac stress and arrhythmia formation, but also hypertension, impaired renal function and the development of insulin resistant diabetes. Tonic chemoreflex activation also destabilizes breathing to increase apnoea incidence, which can then act in a feed-forward manner to propagate this exaggerated chemoreflex state (Dempsey & Smith, 2014). Impairment in baroreflex function, a common feature of these diseases, further amplifies maladaptive chemoreflex effects on cardiovascular sympathetic drive (Zucker *et al.* 2012). Obviously, all of these reflex interactions must be taken into account if one is to devise targeted neuromodulatory therapies to treat the various pathologies delineated above.

Perspectives on disease induced remodelling of neural hierarchy for cardiac control

The thesis presented here is that the cardiac neural hierarchy functions as a distributive processor with multiple nested feedback control loops involving peripheral and central aspects of the autonomic nervous system. It is becoming increasingly evident that control depends first upon the capacity of the neural networks to transduce alterations in regional cardiovascular mechanical and chemical milieus to its various neuronal elements in the reflex control of cardiac motor (adrenergic and cholinergic) neurons. It is further becoming evident that there are inherent and acquired differences in neural network interactions between individuals that impact the progression of cardiac disease to sudden onset (e.g. myocardial infarction) *vs.* chronic disease conditions (e.g. hypertension, diabetes) (Chen *et al.* 2014; Florea & Cohn, 2014; Fukuda *et al.* 2015). It is by understanding the mechanisms by which hierarchical neural networks remodel/adapt in combination with the induced changes in the neural/myocyte interface that rational neuromodulation based therapies can be evolved.

Questions

Remodelling/adaptions in cardiac control with cardiovascular disease

- What are the primary events triggering neural remodelling of peripheral *vs.* central aspects of the cardiac nervous system in cardiovascular disease?
- How do neurochemical and neuroimmune environments of the dorsal root ganglia, spinal networks and supraspinal networks and nuclei contribute to the various cardiac diseases in patients and animal models?
- The mechanisms of typical angina observed in male patients *vs.* atypical angina pectoris as observed in female patients are relatively unknown. What are the pathophysiological differences between sexes?

Autonomic regulation therapy for cardiac disease

Evolution of cardiac disease involves alterations in both cardiac myocytes and the cardiac neuroaxis. Autonomic imbalance, characterized by vagal withdrawal and sustained sympathetic excitation, has been shown to play a significant role in aggravating cardiac disease. Restoration of such balance could, for instance, enhance cardiac function in the presence of cardiac pathologies. Evolving autonomic regulatory therapy (ART; that involves targeting the cardiac neuronal hierarchy) may stabilize such control in the support of cardiac function in disease.

Endogenous ART: exercise. Endurance exercise training represents an effective way to provide relatively safe, non-pharmacological therapy for mitigating subclasses of cardiac diseases (Keteyian *et al.* 2010, 2012; Belardinelli *et al.* 2012), including lethal ventricular arrhythmias (Billman, 2006). It has been known for a long time that exercise training effects parasympathetic (Billman & Kukielka, 2006; Kukielka *et al.* 2006; Billman, 2009) and sympathetic (Billman *et al.* 2006; Holycross *et al.* 2007; Billman, 2009) regulation of the normal heart as well as hearts of patients experiencing myocardial ischaemia. In animal studies, myocardial ischaemia responses have been stratified into (i) susceptible *vs.* (ii) resistant states with respect to ventricular fibrillation induction in pre-training conditions (Billman, 2006). It was found that brief (2 min) periods of coronary artery occlusion can provoke significantly greater increases in heart rate (accompanied by reductions in heart rate variability – an index of cardiac vagal tone) in animals susceptible to sudden cardiac death compared to disease resistant animals (Billman, 2006; Billman & Kukielka, 2006). Exercise training significantly reduced the heart rate response to exercise onset and enhanced (accelerated) return of heart rate to baseline at exercise offset (Billman & Kukielka, 2007). In sedentary animals, heart rate responses to all phases of exercise remain consistent over time (Billman & Kukielka, 2007).

Exercise training improves baroreceptor sensitivity (Liu *et al.* 2001; Zucker *et al.* 2001). In fact, animals that exhibit the greatest increases in heart rate in response to baroreflex challenges proved to be resistant to ischaemia-induced arrhythmias (Billman *et al.* 1982; Billman, 2006). Most importantly, exercise training transformed animals with chronic myocardial infarction that were at high risk of sudden cardiac death to animals that were resistent to ventricular fibrillation even in response to the extreme stress imposed by a second ischaemic event during the end-stages of an exercise stress test (Billman *et al.* 1984; Billman, 2006, 2009). While enhanced parasympathetic activity is a principal manifestation of exercise training, a mitigating of sympathetic tone is also a primary benefit (Liu *et al.* 2001; Zucker *et al.* 2001, 2012).

In order to evaluate the cardiac parasympathetic contribution to the anti-arrhythmic effects of exercise training, studies were repeated after the adminstration of atropine to abolish any exercise training-induced enhancement of cardiac vagal tone. This intervention increased heart rate (Billman & Kukielka, 2006) and provoked reductions in the various indices of heart rate variability but only induced ventricular fibrillation in one of eight trained susceptible dogs (Billman & Kukielka, 2006). Thus, exercise training-induced increases in cardiac vagal tone was not solely responsible for the training-induced protection from ventricular fibrillation. Other factors, including alterations in the β-adrenergic receptor regulation of the heart, must also have contributed to the protection from ventricular fibrillation.

Progression of cardiac disease alters signal transduction in autonomic neural circuits and at the end-terminus of cardiac neural projections (Armour, 2008; Florea & Cohn, 2014; Fukuda *et al.* 2015). β-Adrenergic receptors are a critical aspect of such alterations, reflective of the hyperdynamic sympathetic response (Lefkowitz, 2013; Florea & Cohn, 2014; Fukuda *et al.* 2015). Regular endurance exercise improves β-adrenergic receptor responsiveness in normal (Spina *et al.* 1992; Barbier *et al.* 2004), aged (Mazzeo *et al.* 1995), as well as hypertensive (MacDonnell *et al.* 2005) subjects with little or no change occuring in cardiomyocyte β_1-adrenergic receptor density (Hammond *et al.* 1987; Mazzeo *et al.* 1995; MacDonnell *et al.* 2005). *In vitro* studies indicate that β_2-adrenergic receptor agonists elicited significantly smaller increases in isotonic shortening of ventricular myocytes derived from susceptible dogs after training than those of sedentary animals (Billman *et al.* 2006). *In vivo* studies further demonstrated that before exercise training the β_2-adrenergic receptor antagonist ICI 118,551 significantly reduces peak contractile responses to isoproterenol (isoprenaline) more in susceptible compared to resistant dogs (Billman *et al.* 2006). After exercise training, resistant and susceptible dogs exhibited similar responses to a β_2-adrenergic receptor antagonist (Billman *et al.* 2006). These data indicate that exercise training acts to restore cardiac β-adrenergic receptor balance (by reducing β_2-adrenergic receptor responsiveness) in stabilizing cardiac responsiveness to the stress of exercise. In conjunction with changes in integrated network function within the hierarchy for cardiac control (Zucker *et al.* 2012), these changes in the neural–myocyte interface are fundamental to the cardioprotective effects associated with exercise training.

Vagus nerve stimulation (VNS). *VNS and heart failure.* Vagal stimulation activates multiple signalling pathways that involve (i) afferent-mediated reflexes (Ardell *et al.* 2015; Yamakawa *et al.* 2016) and (ii) direct efferent neuronal targeting of cardiac muscarinic M_2 and M_3

receptors as well as inhibition of pro-inflammatory cytokines (Tracey, 2007; Jänig, 2014*a*) and normalization of nitric oxide signalling (Sabbah, 2011; Sabbah *et al.* 2011*b*). VNS increases the release of the acetylcholine from the cholinergic efferent postganglionic neurons that innervate the mammalian heart. Acetylcholine, in turn, activates cardiomyocyte M_2 muscarinic receptors to induce negative chronotropic, dromotropic and inotropic effects (Levy & Martin, 1979). VNS likewise exerts anti-adrenergic effects mediated within the intrinsic cardiac ganglia (Furukawa *et al.* 1996; McGuirt *et al.* 1997; Randall *et al.* 2003), at the neural–myocyte interface (Levy *et al.* 1966; Levy, 1971; Levy & Martin, 1979) and centrally via afferent mediated changes in sympathetic outflow (Saku *et al.* 2014). Recent data indicate that VNS may also impact myocyte energetics to render myocytes stress resistant (Beaumont *et al.* 2015). Together, such changes restore a physiological balance between energy demands and energy supply of the failing myocardium (Sabbah *et al.* 2011*b*; De Ferrari, 2014; Rhee *et al.* 2015; Buckley *et al.* 2015).

VNS impacts the microenvironment on the heart. First, vagal input inhibits local cytokine release to prevent tissue injury and cell death (Tracey, 2007; Jänig, 2014*a*). These effects appear to be mediated via activation of the α-7 nicotinic acetylcholine receptor (Wang *et al.* 2004) that inhibits the release from macrophages of a mediator of inflammation, namely, high mobility group box 1 (HMGB1) (Wang *et al.* 2004). In fact, long-term VNS in dogs with HF reduces plasma HMGB1 levels along with left ventricle (LV) tissue TNF-α and interleukin-6 (Sabbah, 2011). Secondly, VNS impacts nitric oxide signalling. There are three isoforms of NOS identified to date that are involved in regulation of the heart: endothelial NOS (eNOS), inducible NOS (iNOS) and neuronal NOS (nNOS) (Kelly *et al.* 1996; Feng *et al.* 2002; Mungrue *et al.* 2002; Bendall *et al.* 2004; Nisoli & Carruba, 2006). Coronary artery microembolization-induced HF in canines up-regulates mRNA and protein expression of nNOS (Ruble *et al.* 2010; Sabbah, 2011). In dogs, mRNA and protein expression of myocardial eNOS is significantly down-regulated in HF (Sabbah, 2011), whereas inducible NOS is up-regulated (Ruble *et al.* 2010; Sabbah, 2011). VNS therapy normalizes the expression of nNOS in the failing dog LV and improves iNOS and eNOS expression (Ruble *et al.* 2010; Sabbah *et al.* 2011*a*,*b*). Moreover, exercise training upregulates nNOS and facilitates vagal responsiveness (Danson & Paterson, 2003). These responses are lost in the nNOS knockout mouse, but can be rescued with nNOS gene transfer (Danson *et al.* 2004). Together, these effects serve to augment cardiac energetics, autonomic function and myocardial function leading to improved contractility (Zhang *et al.* 2009*b*; Sabbah *et al.* 2011*a*,*b*; Shinlapawittayatorn *et al.* 2013, 2014; Beaumont *et al.* 2015). VNS has been likewise associated with an improvement in biomarker assessments (e.g. plasma levels of noradrenaline (norepinephrine), angiotensin II and C-reactive protein) (Zhang *et al.* 2009*b*). Finally, VNS can confer a marked survival benefit, at least in the setting of chronic ischaemic heart disease (Li *et al.* 2004).

Based on such preclinical evidence, three clinical studies of VNS have reported the effects of VNS in patients with New York Heart Association (NYHA) classes II–IV chronic reduced ejection HF (HFrEF) (De Ferrari *et al.* 2011, 2014; Premchand *et al.* 2014, 2015; Zannad *et al.* 2015). VNS treatment proved to be feasible, safe and well tolerated, with improvements of quality of life, exercise capacity and left ventricular ejection fraction. Preliminary data from these trials likewise indicated that therapeutic levels of VNS can be delivered from either the right or left vagus (Premchand *et al.* 2014, 2015). To date, two of three reported trials (De Ferrari *et al.* 2011; De Ferrari, 2014; Premchand *et al.* 2014, 2015) have indicated improvements in ejection fraction and decreases in cardiac size with VNS, the third study reporting neutral effects (Zannad *et al.* 2015).

What is clear from these ongoing clinical studies is that the stimulus protocol for VNS is not yet optimized, that patient selection is probably indicated, and the potential interactions with standard of care pharmacological therapies still need to be defined. Recent data further indicate that 'the stronger the better' may not be applicable to VNS therapy, specifically, that therapeutic effects can be achieved without evident bradycardia during on-phase stimulation (Kember *et al.* 2014; Ardell *et al.* 2015). This point, defined as the neural fulcrum, reflects an operating point where bioelectric activation of afferent and efferent projections are balanced such that evoked heart responses are null (Kember *et al.* 2014; Ardell *et al.* 2015) and disease induced imbalances within the cardiac neuronal hierarchy are blunted (Sabbah *et al.* 2011*b*; Beaumont *et al.* 2015).

VNS and ventricular arrhythmias. Animal experiments and clinical studies have demonstrated that VNS imparts anti-arrhythmic protection against both induced and spontaneously occurring ventricular arrhythmias (Billman, 2006; Zipes, 2015). This occurs by both direct (efferent motor) and reflex (afferent) activation of the cardiac neural hierarchy (Kember *et al.* 2014; Ardell *et al.* 2015; Yamakawa *et al.* 2015). Parasympathetic efferent mediated release of ACh that activates end-organ muscarinic receptors (M_2 and M_3) coupled with antagonism of sympathetic efferent outflows to the heart have the net effect to reduce heart rate and prolong APD, reduce APD dispersion and effect ventricular restitution/refractoriness (Brack *et al.* 2007, 2013; Chen *et al.* 2014; Fukuda *et al.* 2015; Herring, 2015). In accord with that, infusion of the non-selective muscarinic receptor antagonist atropine increases the occurrence of induced ventricular fibrillation (VF) (De Ferrari *et al.* 1991).

VNS and atrial arrhythmias. Atrial fibrillation (AF) affects more than three million people a year in the United States (Naccarelli *et al.* 2009). Despite such prevalence, the underlying mechanisms of AF are not fully understood. Current treatments consist of pharmacological therapies that have been combined with localized atrial catheter-based or surgical ablation (Chen *et al.* 2014; Shen & Zipes, 2014). Success rates for such therapy are suboptimum (Cappato *et al.* 2010; Weerasooriya *et al.* 2011). Active neuromodulation therapies for AF represent an emerging therapeutic approach for disease management.

Vagal stimulation has the potential to either increase or decrease the propensity to arrhythmias (Nadeau *et al.* 2007; Lee *et al.* 2013; Chen *et al.* 2014). Higher intensity stimulations tend to increase atrial fibrillation inducibility (Zhang *et al.* 2009*a*,*c*); lower intensity vagal stimulation can stabilize atrial electrical function (Stavrakis *et al.* 2015; Chinda *et al.* 2016). Moreover, recent studies have demonstrated that the anti-arrhythmic effects of VNS can be elicited with minimal adverse effects (Zhang *et al.* 2009*a*; Sheng *et al.* 2011; Zhang & Mazgalev, 2011). It has also been reported that low level VNS therapy suppresses AF induced by cholinergic neuronal activation in ambulatory dogs concomitant with suppression of stellate ganglion hyperactivity (Shen *et al.* 2011; Chinda *et al.* 2016). It has also been hypothesized that obtunding intrinsic cardiac neuronal transduction might represent a mechanistic basis of such benefit (Yu *et al.* 2011; Gibbons *et al.* 2012).

These data indicate that VNS therapy can favourably modify the underlying pathophysiology of ischaemic and non-ischaemic heart disease. It can also prevent the development of malignant ventricular arrhythmias responsible for sudden cardiac death. These benefits apparently are the result of targeting multiple components of the cardiac neuroaxis to: (i) reduce heart rate, (ii) normalize sympathetic inputs to the heart, (iii) suppress pro-inflammatory cytokines; (iv) normalize nitric oxide signalling pathways; and (v) to alter myocyte energetics. Future studies on the efficacy of VNS for cardiac therapeutics should focus on optimization of stimulation parameters, while focusing on patient selection and therapeutic transition where indicated in the standard of care. In view of the fact that VNS engages multiple levels of autonomic control (Ardell *et al.* 2015; Yamakawa *et al.* 2015), future preclinical and clinical studies should be designed to employ the entire cardiac nervous system in order to achieve long-term therapeutic benefits while minimizing off-target side effects.

Autonomic regulation therapy: spinal cord stimulation. Spinal cord stimulation (SCS) has a 20 year history for the treatment of refractory angina pectoris (Mannheimer *et al.* 2002; Foreman & Linderoth, 2012; Zhang *et al.* 2014). Beyond its well characterized anti-anginal effects, SCS exerts multifactorial cardioprotective influences that include suppression of atrial and ventricular arrhythmias (Cardinal *et al.* 2006; Lopshire *et al.* 2009; Gibbons *et al.* 2012) while minimizing apoptotic changes (Southerland *et al.* 2007, 2012) to preserve contractile function (Lopshire *et al.* 2009; Lopshire & Zipes, 2012). A body of preclinical work has demonstrated that SCS fundamentally alters peripheral ganglia neural processing (Armour *et al.* 2002; Ardell *et al.* 2009; Gibbons *et al.* 2012) along with the neural–end-organ interface (Cardinal *et al.* 2006; Southerland *et al.* 2007).

SCS preclinical studies. Cardiac sympathetic afferent neurons transduce the mechanical and chemical milieu of the heart via paravertebral sympathetic ganglia (C8–T6) to the DRG and subsequently to the spinal cord and thereby to higher centres (Foreman, 1999; Armour & Kember, 2004; Foreman & Linderoth, 2012; Yamakawa *et al.* 2016). The spinal cell bodies that convey sympathetic afferent visceral inputs to the brain stem are located in laminae I, V, VII and X in the C8–T9 dorsal horn (Foreman, 1999; Armour & Kember, 2004; Foreman & Linderoth, 2012). Both central and the peripheral reflex processing of such afferent signalling contributes to sympatho-excitation that enhances progression into heart failure (Zucker *et al.* 2012; Florea & Cohn, 2014) and the potential for arrhythmias including sudden cardiac death (Fukuda *et al.* 2015).

SCS impacts autonomic reflexes at multiple levels of the cardiac neuroaxis to minimize sympathetic reflex responses to imposed stress. At the spinal cord itself, SCS induces the release of neuromodulators such as dynorphin that blunt the release of primary afferent related neurotransmitters (such as substance P), and it alters basal activity with sympathetic preganglionic neurons (Ding *et al.* 2008*a*,*b*). Within intrathoracic extracardiac sympathetic ganglia, reflex sympatho-excitation imposed by transient cardiac ischaemic stress is blunted by SCS (Kingma *et al.* 2001; Ardell *et al.* 2009). Likewise, within the intrinsic cardiac nervous system, reflex responses to transient ischaemic stress are blunted by SCS (Foreman *et al.* 2000; Armour *et al.* 2002), the protective effects extending for up to 1 h after SCS offset (Armour *et al.* 2002). The neural memory, subsequent to SCS, has important implications for intermittent SCS, both for cardiac control and angina management (Armour, 2008; Foreman & Linderoth, 2012; Kember *et al.* 2013*b*).

For both intrathoracic extracardiac and intrinsic cardiac ganglia, local circuit neurons appear to be primary targets of SCS therapy (Ardell *et al.* 2009; Gibbons *et al.* 2012). SCS modifies synaptic function without directly altering active and passive transmembrane properties of neuronal somata (Ardell *et al.* 2014). As a consequence of intrathoracic neural targeting, SCS reduces aberrant and heterogeneous electrophysiological activity within the myocardium – even in animal models with chronic ischaemic heart disease (Cardinal *et al.* 2004). Furthermore, in a canine

rapid pace HF model it reduced propensities to ventricular arrhythmias, while improving left ventricular contractile function (Lopshire *et al.* 2009; Lopshire & Zipes, 2012). Finally, the SCS-induced effects on autonomic and end-organ function are positionally dependent. High thoracic SCS (T1–T4) targets thoracic elements for autonomic control of the heart (Foreman *et al.* 2000; Southerland *et al.* 2007; Ardell *et al.* 2009; Lopshire & Zipes, 2014). High cervical SCS (C1–C2) can target both thoracic and visceral autonomic neural networks (Foreman *et al.* 2004; Foreman & Linderoth, 2012; Southerland *et al.* 2012). This has important implications for multi-organ pathologies.

SCS clinical studies. The efficacy of SCS has been found to be optimum when applied pre-emptively (Foreman *et al.* 2000; Southerland *et al.* 2007). Yet, it has demonstrable cardioprotective effects even when applied chronically after the event (Lopshire *et al.* 2009; Ardell *et al.* 2014). Defeat-HF trial (NCT01112579) was a randomized, multicentre, single blind study of 66 patients with systolic HF that failed to show improvement in left ventricular function (Wang *et al.* 2015; Zipes *et al.* 2016). It should be noted that in this study SCS was cycled: 12 h on and 12 h off. In contrast, The Spinal Cord Stimulation Heart study, a multicentre, prospective, pilot trial involving SCS in patients with systolic HF (ejection fraction 20–30% and NYHA class III), reported that continuous T1–T3 SCS (50 Hz for 24 h a day) improved NYHA classification, quality of life, left ventricular end systolic volume and peak oxygen consumption (Tse *et al.* 2015). Such preclinical and clinical studies substantiate the safety of SCS for management of both cardiac arrhythmias and progression into HF. It is evident that additional mechanistic studies are required to delineate the precise mechanisms whereby SCS exerts its effects on (i) central *vs.* (ii) peripheral components of the cardiac neuroaxis in rendering cardiomyocytes stress resistant (Ardell, 2016).

Autonomic regulation therapy: carotid body ablation. Carotid body chemoreceptor activity is elevated in many cardiovascular disease states to negatively impact autonomic balance. Recent studies have demonstrated that the peripheral chemoreceptors, particularly those located in the carotid bodies, play a key role in increasing sympathetic drive in both hypertension and HF (Paton *et al.* 2013; Schultz *et al.* 2015*b*). The maladaptive role of carotid body chemoreceptors in cardiovascular disease is driven primarily by tonic increases in carotid body afferent neuronal discharge (Sun *et al.* 1999; McBryde *et al.* 2013; Schultz *et al.* 2015*a*). Such enhancement of tonic afferent inputs to the brainstem results in a tonic reflex drive that initiaties sympathetic efferent neuronal hyperactivity (McBryde *et al.* 2013; Schultz *et al.* 2015*a*). This response, as a consequence, induces in part the autonomic imbalance characteristic of cardiac disease.

Recent studies have demonstrated that ablation of the carotid bodies improves cardiovascular end points in hypertensive, pre-diabetic and HF animal models (Del Rio *et al.* 2013; McBryde *et al.* 2013; Ribeiro *et al.* 2013; Marcus *et al.* 2014). Carotid body ablation prevents excessive sympathetic motor drive and thus the development of hypertension in rats (Abdala *et al.* 2012; McBryde *et al.* 2013; Ribeiro *et al.* 2013). In spontaneously hypertensive (SHR) rats, carotid body ablation decreases heart rate and increases cardiac baroreflex gain – indicative of the relevance of these bodies to overall cardiovascular control (McBryde *et al.* 2013). In accord with that, hypertensive patients exhibited long-term reductions in systolic arterial pressure (with little change in heart rate) after unilateral carotid body tumour resection (Fudim *et al.* 2015). It has also been shown that carotid body removal in patients results in dissociation of heart rate and blood pressure responses to hypoxia (Niewinski *et al.* 2014). Whereas there was significant attenuation of the hypertensive blood pressure response to hypoxia after carotid body resection, the tachycardia response remained (Niewinski *et al.* 2014). These results suggest that, in humans, the carotid bodies are responsible for ventilatory as well as blood pressure control. In contrast, it appears that chemoreflex control of heart rate might reside primarily with the aortic body chemotransduction.

Thus, carotid bodies play an important role in autonomic control of cardiovascular function – in addition to respiratory instability associated with HF (Del Rio *et al.* 2013; Marcus *et al.* 2014). HF rats (Del Rio *et al.* 2013) and rabbits (Marcus *et al.* 2014) displayed marked shifts in the heart rate variability (HRV) index towards augmented sympathetic tone and reduced parasympathetic tone. In fact carotid body inputs contribute to the cardiac autonomic imbalance and the cardiac sympathetic neuronal excessive activation seen in congestive heart failure (CHF). Chronic carotid body activation has also been shown to contribute to increased arrhythmia incidence in rabbits with HF (Marcus *et al.* 2014) and in rats leads to cardiac remodelling/fibrosis after myocardial infarction (Del Rio *et al.* 2013). Arrhythmias were reversed and cardiac fibrosis prevented by carotid body ablation. In rabbits, carotid body ablation is known to attenuate decreases in the LV ejection fraction while reversing increases in systolic and diastolic ventricular volumes attending chronic cardiac pacing (Marcus *et al.* 2014). Carotid body ablations can also delay progressive left ventricular ejection fraction deterioration after myocardial infarction in rats (Del Rio *et al.* 2013). Taken together, these data indicate the relevant involvement of carotid bodies in controlling cardiac contractile and electrical indices.

Autonomic regulation therapy: carotid sinus stimulation. Baroreceptors can be stimulated in different regions of the arterial tree; the easiest point of stimulation is at

the level of the carotid sinus. While early studies with carotid sinus implants were associated with structural damage to implanted areas (Braunwald *et al.* 1970), evolving biotechnology has overcome such problems. Current devices are implanted with electrodes positioned in the carotid perivascular space around the sinus of the carotid arteries (Sabbah *et al.* 2011*a*; Abraham *et al.* 2015). With time, the interface to the carotid sinus has been miniaturized with unilateral placement (Abraham *et al.* 2015). The premise for such therapy is that stimulation of the peripheral baroreceptor fibres increases afferent activity transduced to the NTS, which is interpreted as an increase in blood pressure. In reflex response to that baroreceptor afferent signal sympathetic efferent are reflexly decreased with a corresponding reflex augmentation in parasympathetic activity, thereby leading to reductions in blood pressure and heart rate. With respect to therapy, minimizing the potential for concurrent activation of the carotid body chemoreceptors with baroreceptor stimulation is imperative given their role in progression of HF by contributing to respiratory instability and oscillatory breathing (changes in tidal volume and respiratory frequency) (Marcus *et al.* 2014).

Baroreceptor activation: preclinical studies. Preclinical studies support proof-of-concept for utilizing bioelectric approaches using carotid sinus stimulation to treat cardiac disease. Such bioelectric stimulation was correlated with reduced plasma angiotensin II and noradrenaline levels and was associated with reduced mortality in a canine model of HF (Zucker *et al.* 2007). Improvements in left ventricular function have been demonstrated in chronic canine HF models with such stimulation. Carotid sinus nerve stimulation improved left ventricle systolic and diastolic function and reduced heart rate compared to untreated heart failure controls (Sabbah *et al.* 2011*a*). Adverse structural remodelling was also mitigated in the treated group.

Autonomic regulation therapy is primarily delivered in an open-loop configuration with an interactive titration phase to the final target levels of therapy (Zipes, 2015; Buckley *et al.* 2015). With the advent of technologies to record relevant biomarkers, the potential to implement closed-loop therapy is now possible. Sunagawa and collegues have recently reported on such methodology, where a sensed arterial pressure signal was utilized to control electrical stimuli applied to the aortic depressor nerve, an aggregate of axons that derive from baroreceptors on the aortic arch (Hosokawa *et al.* 2011, 2012). With such technology they were able to emulate normal dynamic baroreflex function. Further optimization of such approaches would allow for controllable gain and setpoint in a closed-loop setting.

Baroreceptor activation: clinical studies. Clinical trials have been taking place to determine the outcomes of baroreceptor stimulation therapy in both reduced (HFrEF) and preserved (HFpEF) ejection fraction heart failure. The use of implantable carotid sinus stimulator devices (Rheos System), in patients with drug resistant hypertension, showed a sustained blood pressure drop up to 4 years out and improvement in cardiac function (Scheffers *et al.* 2010; Bisognano *et al.* 2011*a*). This was followed by the Rheos Pivotal Trial, a double-blind randomized placebo-controlled trial, that showed 88% maintenance of blood pressure reduction response during a 12 month trial period (Bisognano *et al.* 2011*b*). A recent study investigated baroreflex activation therapy in advanced HFrEF (Abraham *et al.* 2015). Compared to standard medical therapy, patients treated with baroreflex activation therapy showed improved quality of life scores, NYHA classification and N-terminal -pro-brain natriuretic peptide (NT-proBNP); however, it did not show any significant change in left ventricular function (Abraham *et al.* 2015). Thus the use of carotid sinus nerve stimulation to modulate baroreflex control mechanisms has received proof-of-concept safety approval for therapy in heart disease. Future studies will be required to improve electrode interfaces, stimulus protocols, and the potential for closed-loop feedback.

Autonomic regulation therapy: renal denervation. *Preclinical studies.* Afferent signalling from somatic, visceral and thoracic sites contribute to integrated cardiac control. Owing to the central role that the renal circulation plays in volume regulation and in control of the renin–angiotension system, autonomic regulation therapy targeted to the renal circulation has received extensive interest. Targeting renal arterial nerves has been accomplished by catheter ablation delivered to the renal arteries under fluoroscopic and/or electro-anatomic mapping guidance. For instance, renal sympathetic axonal modulation in a rat model with compensated high output HF resulted in attenuated sodium excretion after sodium loading (Villarreal *et al.* 1994). In rats with chronic myocardial infarction, renal neural ablation increased sodium excretion, decreased LV filling pressure and improved left ventricular function (Nozawa *et al.* 2002). In a rabbit model of pacing induced HF, renal denervation modified angiotensin II receptor expression and preserved renal function (Clayton *et al.* 2011). In the canine model of pacing-induced HF, renal nerve ablation reduced circulating angiotensin II, aldosterone, brain natriuretic peptide, endothelin-1, transforming growth factor β (TGF-β) and renalase in conjunction with reduced ventricular substrate remodelling compared to untreated controls (Dai *et al.* 2014; Zhao *et al.* 2014). With such renal sympathetic modulation, substrate and electrical remodelling were correspondingly mitigated and inducibility of ventricular fibrillation decreased (Guo *et al.* 2014).

Renal axonal modulation: clinical studies. Small case studies have demonstrated a reduction of ventricular arrhythmia potential post renal nerve denervation (Bradfield *et al.* 2014; Remo *et al.* 2014). Although the beneficial effect for arrhythmia burden did not specifically improve left ventricular function, frequent lethal ventricular arrhythmias in the setting of LV dysfunction were attenuated (Bradfield *et al.* 2014; Remo *et al.* 2014). There is mounting evidence that suggests benefits in preserved ejection fraction, probably as a result of the anti-hypertensive effects of such therapy, thereby resulting in less structural remodelling (Brandt *et al.* 2012). What is clear from recent experience with renal ablation for hypertension, Symplicity HTN-1 to HTN-3 (Krum *et al.* 2009; Esler *et al.* 2010; Kandzari *et al.* 2012; Bhatt *et al.* 2014; Blankestijn *et al.* 2015), is that effective implementation of any type of autonomic regulation therapy requires an objective index for efficacy of treatment delivery. For Symplicity HTN-3, suboptimum nerve ablation was probably responsible in part for trial failure (Blankestijn *et al.* 2015; Epstein & de Marchena, 2015).

Autonomic regulation therapy: cardiac afferent targets. Several studies have now shown that there is an enhanced input from cardiac sympathetic afferents in experimental CHF. Wang *et al.* (Wang & Zucker, 1996) carried out experiments in anaesthetized dogs with pacing-induced CHF. Epicardial surface or left atrial administration of bradykinin or capsaicin enhanced renal sympathetic nerve activity and arterial pressure in the CHF state, compared to sham operated animals. Furthermore, in CHF animals cardiac afferent axons that transduce local epicardial application of capsaicin show enhanced discharge (Zucker *et al.* 1996). Perhaps the most convincing evidence for enhanced tonic input from cardiac sympathetic afferent neurons to the spinal cord is the observation that in anaesthetized dogs with the vagi sectioned application of epicardial lidocaine resulted in a significant reduction in renal sympathetic nerve activity that is not seen in sham animals (Zucker *et al.* 1996). Furthermore, in the CHF state the cardiac sympathetic afferent reflex has been shown to be modulated by central angiotensin II through the AT_1 receptor and oxidative stress (Zhu *et al.* 2002, 2004*a*,*b*; Wang *et al.* 2007, 2008; Gao *et al.* 2008), thus indicating defects at multiple sites in the neuraxis in animals with CHF.

Because the sensory endings of DRG neurons release a variety of neuropeptides (Steinhoff *et al.* 2014), sensitization of these endings in the diseased heart may have a profound effect on their potential to transduce local ventricular function and to initiate an inflammatory process that participates in the cardiac remodelling following myocardial infarction. In recent work by Wang *et al.* (Wang *et al.* 2014) the role of cardiac sympathetic afferent neurons in such sympatho-excitation, baroreflex function and cardiac remodelling post MI has been demonstrated. In fact, many cardiac sympathetic afferent neurites express the TRPV1 receptor that is normally activated by agents such as capsaicin that participate in pain transduction (Wu & Pan, 2007).

Employing the highly selective TRPV1 agonist resiniferatoxin (RTX) (Lee *et al.* 2012) Wang *et al.* were able to ablate sensory neurites on or near the epicardium at the time of coronary artery ligation (Wang *et al.* 2014). Twelve weeks later, rats were evaluated for sympathetic function and cardiac remodelling. At this time there was a clear reduction in baseline cardiac function while renal sympathetic neuronal outflow response to epicardial application of capsaicin was completely abolished. In fact, there was a reduction in global sympathetic tone, as evidenced by reduced noradrenaline excretion. This intervention also reduced myocardial fibrosis in regions remote from the scar. Furthermore, markers of the fibrotic process such as fibronectin and TGF-β receptor expression were normalized in these RTX treated animals – a finding of considerable clinical relevance. As such, there was improvement in LV diastolic function (the slope of the end-diastolic pressure–volume relationship (EDPVR) was decreased) and increased responsiveness to isoproterenol in the presence of maintained basal LV systolic function. The fundamental premise here is that aberrant afferent activation is central to the evolution of cardiac disease and that mitigation of that afferent signal is an emerging therapeutic target.

Decentralization of the peripheral cardiac nervous system. As depicted above, patients with structural heart disease are known to be at risk of ventricular arrhythmias that progress to sudden cardiac death (SCD) (Vaseghi & Shivkumar, 2008). They are usually treated with medication and/or catheter ablation (Florea & Cohn, 2014; Fukuda *et al.* 2015). Despite that, subsets of patients remain who are refractory to these therapies and continue to experience incessant ventricular arrhythmias with a high risk for SCD. In that population, direct targeting of selective nodes of the autonomic nervous system is emerging as an effective adjunct therapy (Vaseghi & Shivkumar, 2012). This includes surgical approaches to the cranial thoracic paravertebral chain to modulate autonomic imbalance and reduce cardiac arrhythmias (Schwartz, 2014). In fact, resection of the stellate caudal to the T4 paravertebral ganglia bilaterally has been shown to effectively impart anti-arrhythmic effects in patients with ventricular tachycardia that are refractory to other forms of therapy (Ajijola *et al.* 2012; Vaseghi *et al.* 2013, 2014). For further details on this critical neuromodulation methodology, see the companion White Paper by Shivkumar *et al.* (2016).

Questions

Autonomic regulation therapy for cardiovascular disease

- What are the preferential sites for ART in the cardiac neuraxis? Do they differ depending on the aetiology of the cardiovascular disease? How does it relate to patient selection?
- What is/are the critical neural targets for ART – afferent, efferent or local circuit neurons – intrathoracic *vs.* central?
- What are the appropriate biomarkers to assess the efficacy of differing forms of ART in the short-, mid- and long-term?

Concluding perspectives

Neurocardiology is based on the premise that cardiac control must be evaluated in the context of the end-organ substrate, interdependent interactions within multiple levels of the cardiac nervous system (peripheral and central), and relevant neuro-humoral responses. It is clear that there are inherent differences that impact the autonomic response to acquired stressors and that such differences are critical to final outcomes. Imbalances in autonomic evoked responses, while beneficial in the short term, are often hyper-dynamic and ultimately contribute to the evolution of cardiac pathology. What is also clear is that by a mechanistic understanding of these interactions, novel neural based therapeutic targets have emerged to restore a more balanced autonomic response. Since cardiac pathology is multifaceted, it is also likely that the optimum neural target will be influenced by the underlying stressor (e.g. myocardial infarction, hypertension). As our understanding of where to target emerges, there is also a need to identify appropriate biomarkers such that closed-loop autonomic regulation therapies can evolve. The ultimate goal is to work with endogenous control systems, rather than in opposition to them, to optimize outcomes.

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Additional information

Competing interests

None declared.

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