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Differential cardiac responses to unilateral sympathetic nerve stimulation in the isolated innervated rabbit heart

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ABSTRACT

The heart receives both a left and right sympathetic innervation. Currently there is no description of an *in vitro* whole heart preparation for comparing the influence of each sympathetic supply on cardiac function. The aim was to establish the viability of using an *in vitro* model to investigate the effects of left and right sympathetic chain stimulation (LSS/RSS). For this purpose the upper sympathetic chain on each side was isolated and bipolar stimulating electrodes were attached between T2–T3 and electrically insulated from surrounding tissue in a Langendorff innervated rabbit heart preparation ($n=8$). Heart rate (HR) was investigated during sinus rhythm, whilst dromotropic, inotropic and ventricular electrophysiological effects were measured during constant pacing (250 bpm). All responses exhibited linear increases with increases in stimulation frequency (2–10 Hz). The change in HR was larger during RSS than LSS ($P<0.01$), increasing by 78 ± 9 bpm and 49 ± 8 bpm respectively (10 Hz, baseline; 145 ± 7 bpm). Left ventricular pressure was increased from a baseline of 50 ± 4 mm Hg, by 22 ± 5 mm Hg (LSS, 10 Hz) and 4 ± 1 mm Hg (RSS, 10 Hz) respectively ($P<0.001$). LSS, but not RSS, caused a shortening of basal and apical monophasic action potential duration (MAPD90). We demonstrate that RSS exerts a greater effect at the sinoatrial node and LSS at the left ventricle. The study confirms previous experiments on dogs and cats, provides quantitative data on the comparative influence of right and left sympathetic nerves and demonstrates the feasibility of isolating and stimulating the ipsilateral cardiac sympathetic supply in an *in vitro* innervated rabbit heart preparation.

1. Introduction

The sympathetic fibres, which innervate the heart, arise from the level of T1–T5 of the thoracic vertebrae and are distributed through the inferior cervical, middle cervical, stellate and mediastinal ganglia (Randall and Rohse, 1956; Hopkins and Armour, 1989; Armour, 2008) on both right and left sides. The postganglionic sympathetic fibres converge at the cardiac plexus and from there innervate the sinoatrial node (SAN), the atrioventricular node (AVN) and the atrial/ventricular myocardium (Hopkins and Armour, 1984; Armour, 2008). The effects of denervation (stellectomy) or sympathetic nerve stimulation on the contractile performance and electrical properties of the myocardium are described both in traditional *in vivo* studies in dog and cat (Sonnenblick et al., 1963; Vassalle et al., 1968; Spear and Moore, 1973; Wilde et al., 2008), and in isolated heart preparations utilising adrenoceptor agonists (Feinberg and Katz, 1958). The known effects of increased sympathetic activity are an increase in heart rate (HR, positive chronotropy), greater force of contraction (positive inotropy), enhanced relaxation (positive lusitropy) and reduced delay at the AVN (positive dromotropy). Evidence from studies in anaesthetised dogs and cats and in conscious animals indicates that there are differences in the functional and electrophysiological responses of the heart to unilateral left and right sided nerve stimulation or denervation (Anzola and Rushmer, 1956; Randall and Rohse, 1956; Yanowitz et al., 1966; Geis and Kaye, 1968; Haws and Burgess;, 1978; Ardell et al., 1988; Priori et al., 1988; Zaza et al., 1991). This heterogeneity has been well documented in extensive studies on anaesthetised dogs in Randall's lab and strongly indicates a dominant influence of right sympathetic innervations on rate whilst the left sympathetic innervation primarily influences ventricular contractility (Randall and Rohse, 1956; Randall et al., 1963; Randall et al., 1968; Randall, 1984; Ardell et al., 1988). In accord with these data, the left sympathetic has a greater influence on ventricular action potential duration and repolarisation and as a consequence is strongly implicated in the generation of left ventricular arrhythmias, as has been well documented by studies in anaesthetised cats (Zaza et al., 1991) and in conscious animals (Schwartz et al., 1984).

Studies on the autonomic control of cardiac function have traditionally utilised *in vivo* models where autonomic nerves can be stimulated

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directly, or *in vitro* preparations using mimetics (both natural and synthetic) of sympathetic or parasympathetic neurotransmitters. Both approaches have a number of advantages and the use of the former has clearly demonstrated a distinct differential influence of left and right neural innervations on cardiac function. However, for the purposes of detailed analysis of the neuro-myogenic mechanisms there may be a number of draw-backs to each approach. *In vivo* studies are confounded by circulating hormones and cardiac afferent induced reflexes as well as changes secondary to haemodynamic reflexes. Furthermore the level of background activity in the sympathetic nerves can vary considerably in conscious or anaesthetised animals. Such factors may have contributed to a high degree of variability in published data to date (Yanowitz et al., 1966). *In vitro* preparations utilising pharmacological and chemical agonists, such as isoprenaline, lack the targeted approach of direct nerve stimulation, do not take into account the natural heterogeneity of innervation in the heart and suffer from the toxic consequences of many of these agents (Rona et al., 1959; Rona, 1985).

In 2001, our group developed an *in vitro* dual innervated (sympathetic and parasympathetic divisions) Langendorff perfused rabbit heart preparation which circumvents circulatory influences and underlying autonomic tone whilst preserving the effects of autonomic innervation to the heart. This preparation has since been extensively characterised demonstrating classical responses to both sympathetic and parasympathetic nerve stimulation and has yielded a number of important observations on their actions and new mechanistic insights (Ng et al., 2001; Brack et al., 2004; Brack et al., 2006; Brack et al., 2007; Mantravadi et al., 2007; Ng et al., 2007; Brack et al., 2009; Ng et al., 2009; Brack et al., 2010). These studies with the dual innervated rabbit preparation have relied upon direct stimulation of sympathetic neurones within the spinal cord that supply both right and left stellate ganglia and as such do not account for effects of differential sympathetic outflow arising from the left and right sympathetic chains. Therefore, the influence of right and left cardiac sympathetic supply has not been tested in this model, and this is necessary if we are to use the model to investigate the role of heterogeneous sympathetic innervation in cardiac arrhythmogenesis.

The aim of this study was to modify our original innervated isolated heart preparation to allow for unilateral sympathetic chain stimulation and to quantify the differential functional effects of the two cardiac sympathetic nerve projections. The data confirm previous conclusions in dog and cat on the functional heterogeneity of the left and right cardiac sympathetic nerves but also provide new quantitative information on the magnitude of the changes induced by either left or right preganglionic cardiac sympathetic nerves on dromotropism, inotropism and electrophysiological parameters of the left ventricle. It reports the first functional evidence of the heterogeneity of sympathetic innervations in the rabbit heart. Additionally, the successful further development, achieving separate right and left sympathetic nerve stimulation in this isolated innervated rabbit heart preparation provides an essential step to enable a more controllable and quantifiable approach to studying mechanisms contributing to left ventricular arrhythmogenesis and changes in contractility.

2. Materials and methods

2.1. Experimental techniques

All procedures were undertaken after local ethics approval at the University of Leicester and were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and the Guide for the Care and Use of Laboratory Animals Published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1985).

2.2. Isolation and perfusion of hearts with intact autonomic innervations

The innervated isolated rabbit heart preparation was isolated and perfused as previously described (Ng et al., 2007). Briefly adult male New Zealand White rabbits (1.7–2.5 Kg, n = 8) were pre-medicated with ketamine (Ketaset, 10 mg/kg, Fort Dodge, Southampton, United Kingdom), medetomidine hydrochloride (Sedator, 0.2 mg/kg, Dechra, Shrewsbury, United Kingdom) and butorphanol (Torbugesic, 0.05 mg/kg, Fort Dodge Southampton, United Kingdom) (i.m.). 15–20 min later anaesthesia was induced by propofol (i.v., Rapinovet, Schering-Plough Animal Health, Milton Keynes, United Kingdom) and maintained for the remainder of surgery. Animals were intubated (tracheotomy) and breathing was regulated using a small animal ventilator at 60 breaths per minute of room air. The common carotid arteries were identified and isolated and the vagus nerves partially dissected free at the mid-cervical level. A midline incision exposed the rib cage allowing for dissection of the pectoral muscles and the isolation of the subclavian vessels. Animals were killed with an overdose of sodium pentobarbitone (Sagatal, Rhone Merieux, Harlow, UK; 800 mg, i.v.) together with 1000 U heparin (i.v.). The thoracic cavity was opened and the anterior portion of the ribcage removed exposing the mediastinal contents. The descending aorta was identified and cannulated following which hearts were perfused with ice cold Tyrode solution, to reduce metabolic rate. The vertebral column was cut at the level of the 12th thoracic and 1st cervical vertebrae and the preparation was dissected from the surrounding tissues for Langendorff perfusion.

2.3. Langendorff perfusion

The preparation was perfused at a constant rate of 100 ml/min in Langendorff mode with an oxygenated Tyrode solution of the following composition (mM): Na^+ 138, K^+ 4, Ca^{2+} 1.8, Mg^{2+} 1.0, HCO_3^- 24, $H2PO_4^-$ 0.4, Cl^- 121, glucose 11 (pH 7.4 — bubbling with O_2/CO_2 — 95/5%, temperature maintained at 37 °C). A catheter (3F, Portex, Kent, United Kingdom) was inserted through the apex of the left ventricle for drainage of fluid returning to the left ventricle (LV) from the thebesian veins. A fluid filled latex balloon was inserted into the left ventricle, through an incision in the left atrial appendage, and used for the recording of left ventricular pressure (LVP) through a pressure transducer (MLT0380/D, ADI instruments Ltd, Charlgrove, United Kingdom). Aortic perfusion pressure was recorded through a second pressure transducer in series with the perfusion system.

2.4. Left and right sympathetic chain isolation and stimulation

The left and right sympathetic chains run adjacent to the spinal column and were first visualised through careful dissection of the connective tissues at the level of the 4th or 5th ribs with a Zeiss dissecting microscope. Following this the chains were dissected up to the level of the 1st and 2nd intercostal space close to the upper intercostal artery, which runs inferiorly to the chains in this region, and was ligated and cut. A drain was inserted through the thoracic wall to aid fluid drainage in this region. A pair of custom made silver electrodes (Advent Research Materials, Oxford, United Kingdom: 0.5 mm diameter) was placed around each chain so that they spaced from just below the 2nd and up to the 3rd rib ensuring adequate clearance from the surrounding connective tissues. These electrodes were then electrically isolated from the surrounding tissues using quick setting dental cement (Klass 4 dental cement, Klass 4 dental supplies). Fig. 1A shows a diagrammatic representation of the experimental model and Fig. 1B a representation of the electrode positioning on the sympathetic chain.

Constant voltage stimulators (SD9, Grass Instruments, Astro-MedInc., Slough, United Kingdom) were used to stimulate the left and right sympathetic chains separately. Once the sympathetic chains were isolated and the dental cement had set, each sympathetic chain was stimulated using incremental voltages from 0.1 V to 3 V at 5 Hz (2 ms pulse width) until there was an increase in *both* LVP (of 3–4 mm Hg) and heart rate (of 3–4 bpm). The voltage that caused an increase in both parameters was defined as the threshold voltage. The remainder of the protocols was performed at twice the threshold voltage.

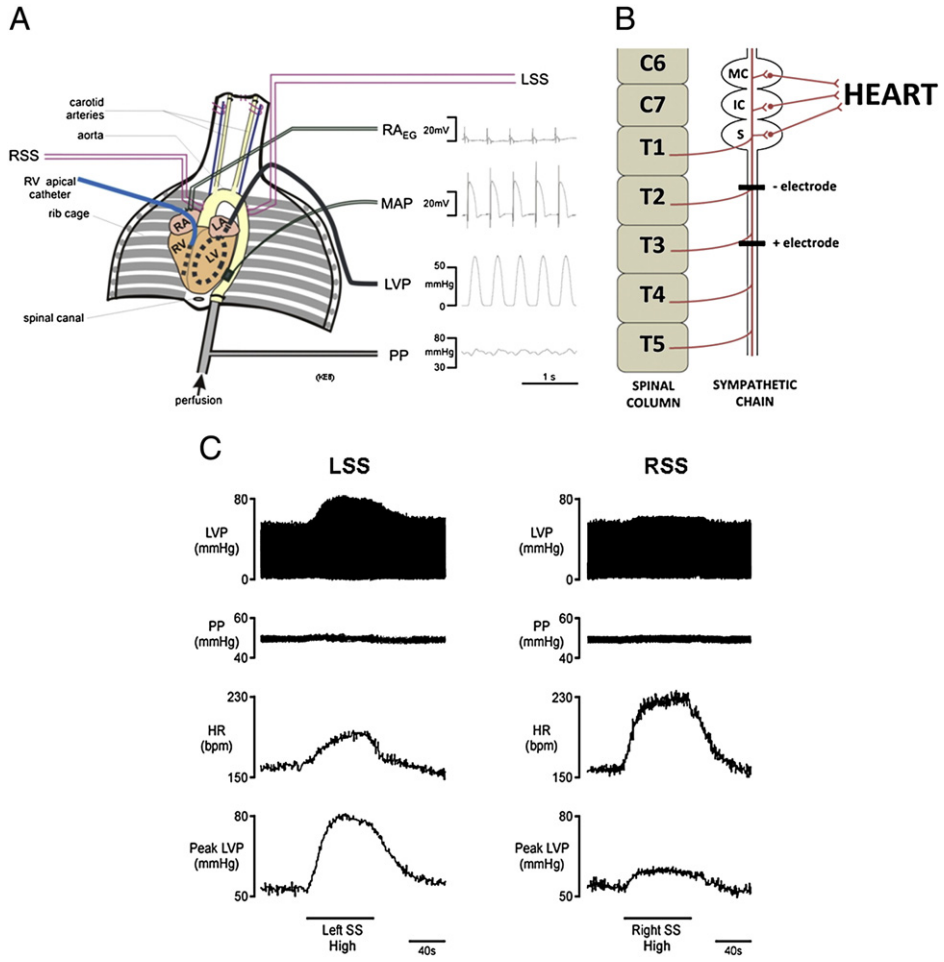

Fig. 1. Model diagram and preliminary data. Diagrammatic representations of the isolated rabbit heart preparation with intact dual autonomic nerves (A) and electrode positioning on the left sympathetic chain (identical placement on right side) (B). Preliminary data demonstrating the differential effects of left and right sympathetic stimulation (SS) on left ventricular pressure (LVP), perfusion pressure (PP), heart rate (HR) and peak LVP during sinus rhythm (C). LA & RA: left and right atrium, LV & RV: left and right ventricle, LSS & RSS: left and right sided sympathetic stimulation, MC: middle cervical ganglion, IC: inferior cervical ganglion, S: stellate ganglion.

2.5. Cardiac electrical recording and pacing

A pair of platinum electrodes (Grass Instruments, Slough, United Kingdom) were inserted into the right atrial (RA) appendage for recording of atrial electrograms. The recording of atrial electrograms during right ventricular (RV) pacing allows retrograde ventriculo-atrial conduction analysis to be made. The same wires were used for atrial pacing to examine antegrade atrio-ventricular conduction (see below). Custom-made suction electrodes were used to record monophasic action potentials (MAPs), as previously described (19), from the epicardial surface of the left ventricular free wall simultaneously at the apex and the base and analysed using custom made software (Dr Francis Burton, Glasgow University, Glasgow, UK).

2.6. Protocols

Left and right sympathetic stimulations (left SS and right SS respectively) were carried out at 2 Hz (Low), 5 Hz (Medium) and 10 Hz (High) frequencies following the protocol below with a minimum period of rest between stimulation of 3 min:

2.6.1. Chonotropic effects of left and right SS

Paravertebral chains were stimulated during intrinsic sinus rhythm to determine effects on chronotropy (HR). To analyse the dynamic change in HR, the duration from the start of nerve stimulation to a 1% increase in HR was also measured.

2.6.2. Ventricular effects of left and right SS

2.6.2.1. Inotropy. Hearts were paced via the right ventricle at 250 bpm to determine effects of SS on LVP in order to assess inotropic changes. LVP was measured at baseline and during the plateau phase of the response to SS (*i.e.* during the new steady state). To analyse the dynamic change in LVP, the duration from the start of nerve stimulation to a 1% increase in LVP was also measured.

2.6.2.2. Ventricular electrophysiological parameters. Apical and basal MAPs were recorded and effects of sympathetic nerve stimulation were measured at 90% decay of the MAP voltage ($MAPD_{90}$) at baseline and steady state during constant pacing (250 bpm).

2.6.3. Dromotropic effects of left and right SS

RA electrograms were recorded along with all other parameters to measure dromotropic changes during constant ventricular pacing as measured by retrograde ventriculo-atrial conduction. This was obtained by measuring the delay from the RV pacing spike to the atrial electrogram.

The dromotropic effects during left and right SS were also investigated during pacing at the right atrium (250 bpm). This gives a measure of antegrade atrioventricular conduction and was calculated as

the time delay from the atrial stimulation spike to the beginning of the monophasic action potential measured from the LV free wall.

2.7. Signals measurements and analysis

All pressure and electrical signals obtained from the preparation were recorded with a PowerLab 16/30 system (ADInstruments Ltd) and digitised at 1 kHz using Chart software (ADInstruments Ltd) with the data stored and displayed on a personal desktop computer. The left SS and right SS stimulus signals were also recorded on the same system as well as the timing and duration of the stimuli. Instantaneous intrinsic sinus heart rate and peak LVP were measured throughout the experiment as a guide for maximal and steady state effects. Heart rate measurements were taken from an average of 10 cardiac cycles, whilst all other signals were averaged during constant ventricular or atrial pacing from a 30 second period during steady state. Within each branch of SS, one-way ANOVA was used to compare the parameters between baseline values and at steady state during SS, with Bonferroni post-hoc tests used to determine the comparisons that were significantly different. Two-way ANOVA was used to analyse the changes in heart rate to demonstrate 1) any differential effects of left and right sympathetic stimulation (*i.e.* left *vs.* right or laterality), 2) the effects of frequency and 3) any interaction between frequency and laterality on these changes. $P<0.05$ was considered significant. All figures and illustrations within this manuscript were produced using OriginLab 8.5 (Northampton, USA) and Corel DRAW Graphics Suite 11 (Maidenhead, United Kingdom).

3. Results

Fig. 1B illustrates the typical changes seen in HR and LVP during concurrent high intensity left and right SS. The differential effects of left and right SS are clearly demonstrated. Left SS produced more prominent changes in LVP when compared to right SS whereas there was a greater increase in HR with right SS when compared with left SS. The effects of left and right SS on HR and LVP are quantified independently (below) using the previously described experimental protocols (see subheading 2.6).

3.1. Effect of left and right sympathetic stimulation on heart rate

Data illustrating the effect of left and right SS on beat-by-beat intrinsic sinus heart rate and the percentage change in heart rate from the start of nerve stimulation in a typical experiment is shown in Fig. 2A–D. Left SS and right SS induced a monotonic increase in HR to a plateau that was maintained until the cessation of nerve stimulation (Fig. 2A–B). The change in HR during SS was dependent upon the frequency of nerve stimulation demonstrating a larger change during higher frequency SS (Fig. 2A–B). HR changes were greater during right than during left SS (Fig. 2A–B). Similarly the dynamic change in heart rate (*i.e.* time taken for a 1% change from baseline values (lag)) was dependent upon the frequency of stimulation and location of SS (*i.e.* faster changes during right SS), as shown in Fig. 2C–D.

Mean data confirming these findings are shown in Fig. 3. Both left (Fig. 3A) and right SS (Fig. 3B) induced significant increases in HR at all frequencies. HR responses were dependent upon the frequency of stimulation (Fig. 3C, $P<0.01$) with right SS producing a larger increase in HR compared to left SS (Fig. 3C, $P<0.01$). The HR lag time was found to be shorter with right SS ($P<0.001$) and with higher frequency of nerve stimulation in both left and right SS (Fig. 3D, $P<0.001$). Interaction test was significant ($P<0.05$) demonstrating that frequency of SS had an effect on laterality, with data suggesting that differences between left and right SS were more obvious at low frequency of stimulation.

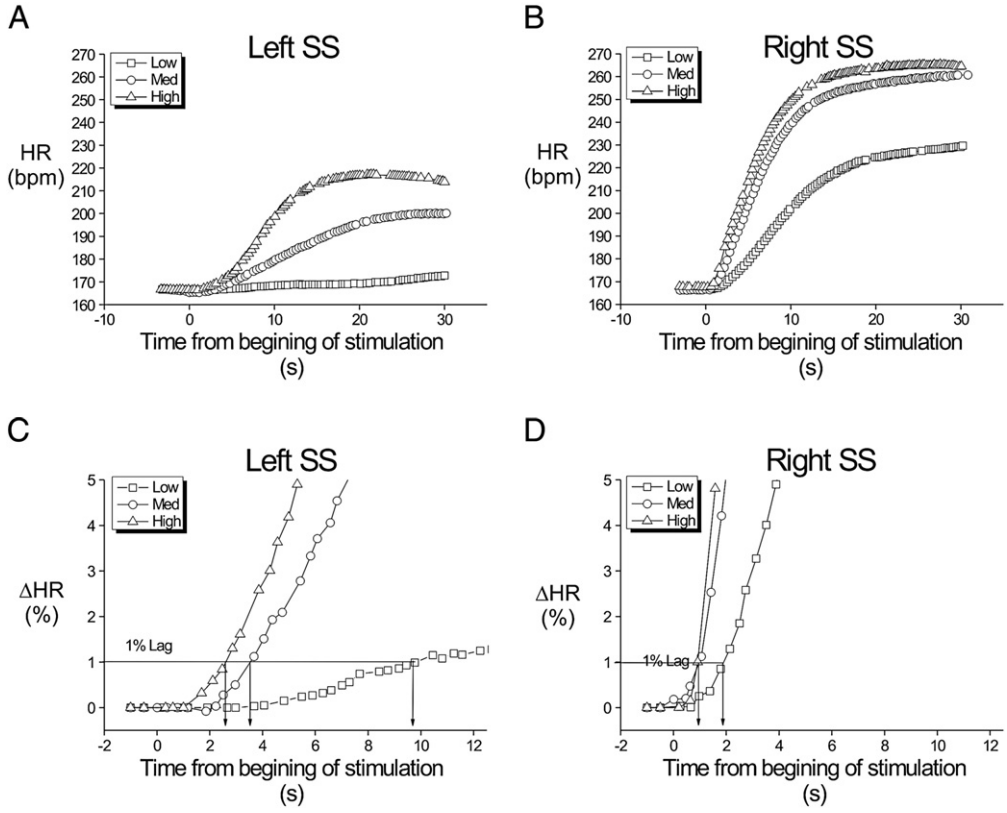

Fig. 2. Raw data illustrating the effect of unilateral sympathetic stimulation on heart rate during sinus rhythm. A–B) The effect of left and right sympathetic stimulation (SS) on heart rate (HR) at low (2 Hz), medium (5 Hz) and high frequencies of stimulation (10 Hz) from a single representative experiment. C–D) The percentage increase in HR from baseline values at low, medium and high frequency LSS and RSS.

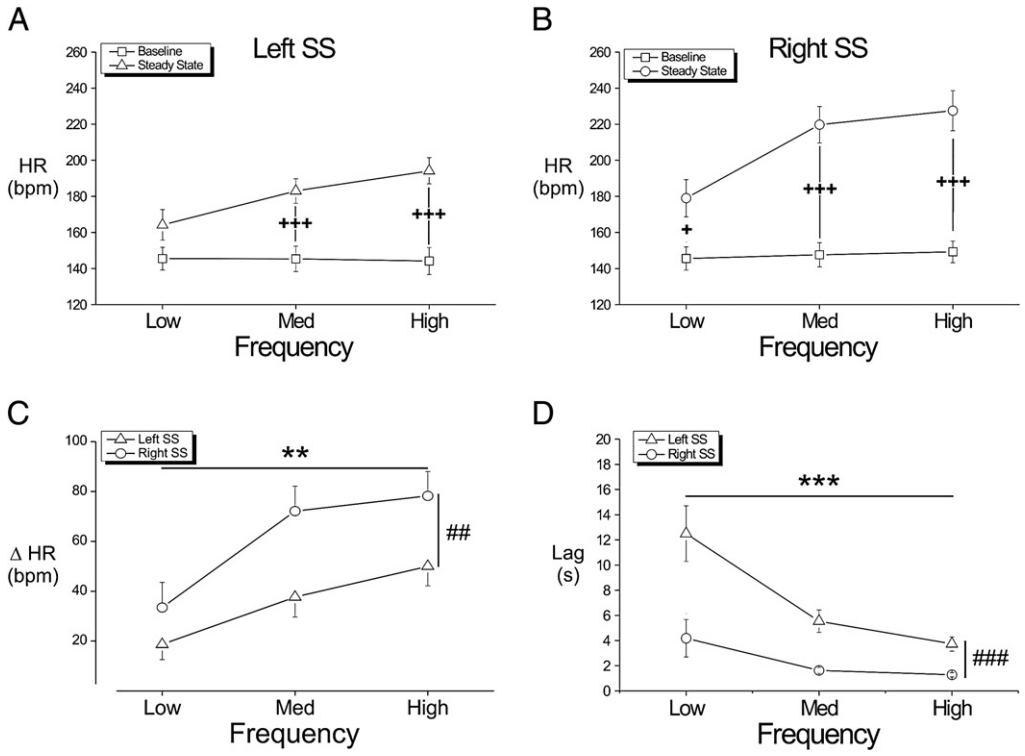

Fig. 3. Effect of unilateral sympathetic stimulation on heart rate during sinus rhythm. Mean data illustrating baseline and steady state heart rate (HR) during left (A) and right (B) sided sympathetic stimulation (SS). The change in HR from baseline (C) and lag time (time taken to achieve a 1% increase in HR, D). Data represent mean ± SEM, n = 8. Baseline *vs.* steady state: $^{+}P<0.05$, $^{+++}P<0.001$; frequency: $^{**}P<0.01$, $^{***}P<0.001$; left *vs* right: $^{\#\#}P<0.01$, $^{\#\#\#}P<0.001$.

3.2. Effect of left and right sympathetic stimulation on LV functional and electrophysiological parameters

3.2.1. Functional parameters

3.2.1.1. LVP. Effects of left and right SS on ventricular inotropy were examined during constant ventricular pacing. Raw data illustrating the effect of left and right SS on continuous perfusion pressure, left ventricular pressure and peak LVP in a typical experiment is shown in Fig. 4. LVP responses to left SS were bi-phasic consisting of an initial increase to a peak response followed by a gradual decline to a new plateau (above that of baseline). Right SS was associated with less inotropic enhancement.

Mean data on LVP are illustrated in Fig. 5A–D. Both left (Fig. 5A, $P<0.001$) and right SS (Fig. 5B, $P<0.001$) produced a frequency dependent increase in LVP (Fig. 5C, $P<0.05$). The increase in LVP in response to left SS was significantly greater than that seen with right SS (Fig. 5C, $P<0.001$). The time taken to reach a 1% change in LVP response (lag time) was also dependent on stimulation frequency during both left and right SS (Fig. 5D, $P<0.05$). The lag time in the LVP increase was significantly shorter during left SS than that seen during right SS (Fig. 5D, $P<0.05$).

3.2.1.2. dP/dt. In keeping with the patterns noted in LVP, the rate of LVP development (dP/dt_{max}) was increased to a greater extent during left SS than during right SS (Fig. 6A–C, $P<0.05$). A greater rate of ventricular pressure relaxation (dP/dt_{min}) was noted during left SS *vs.* right SS (Fig. 6D–E, $P<0.05$).

3.2.1.3. LV end diastolic pressure and perfusion pressure. Left ventricular end diastolic pressure and aortic perfusion pressure did not change significantly during either left SS or right SS stimulation (data not shown).

3.2.2. Electrophysiological parameters

An example of the effect of left SS and right SS on left ventricular MAPs recorded from the apex and basal region is illustrated in Fig. 7A. Left SS caused a frequency dependent shortening of both basal and apical $MAPD_{90}$ (Fig. 7B–C). Right SS was not associated with any significant change in $MAPD_{90}$ in either basal or apical regions.

3.3. Effect of left and right sympathetic stimulation on antegrade and retrograde conduction through the atrioventricular node

Fig. 8 illustrates the effect of left SS and right SS on both retrograde (Fig. 8A) and antegrade (Fig. 8B) conduction through the AV node in a typical experiment. Both left and right SS were associated with a frequency dependent shortening of conduction time through the AVN during ventricular (retrograde conduction) pacing (Fig. 8B–C $P<0.05$). Comparison of left and right SS reveals that left SS resulted in a greater rate dependent shortening of retrograde conduction time through the AVN (Fig. 8C) than during right SS. Similar results were obtained during atrial pacing (data not shown).

4. Discussion

It is acknowledged that the existence of a functional heterogeneity between right and left sympathetic supplies to the heart is an old observation dating from the latter part of the 19th century (Boehm and Nussbaum, 1875). More recent experiments conducted in dogs and cats, have confirmed and expanded this understanding (Randall, 1984; Schwartz et al., 1984). However, there has been no clear quantification of the comparative actions of the two nerve supplies. To our knowledge the present study is the first description of the differential functional effects of increasing levels of induced activity in left and right sided sympathetic innervations on cardiac contractile performance and electrophysiological properties, and furthermore the first description in the isolated rabbit heart. We are aware that this is a

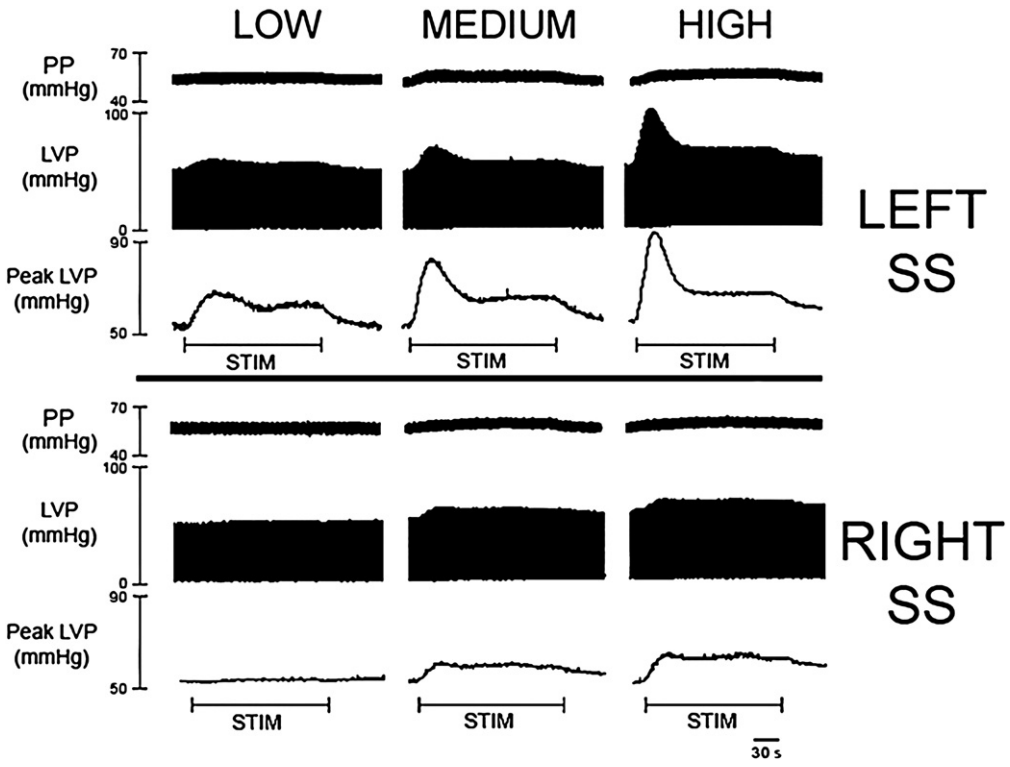

Fig. 4. Raw data trace of the effect of unilateral sympathetic stimulation on left ventricular pressure during constant pacing. Effect of left and right sided sympathetic stimulation (SS) on left ventricular pressure (LVP), perfusion pressure (PP) and peak LVP at low (2 Hz), medium (5 Hz) and high (10 Hz) frequencies of stimulation during right ventricular pacing 250 at bpm.

small step, but such quantitative data are a necessary prerequisite for future *in vitro* studies, using the isolated innervated rabbit heart model, into the role of heterogeneous sympathetic innervation in the development of malignant cardiac arrhythmias. Our data show that nerves arising from the left sympathetic paravertebral chain have a greater influence on LV contractility, LV MAPD and conduction through

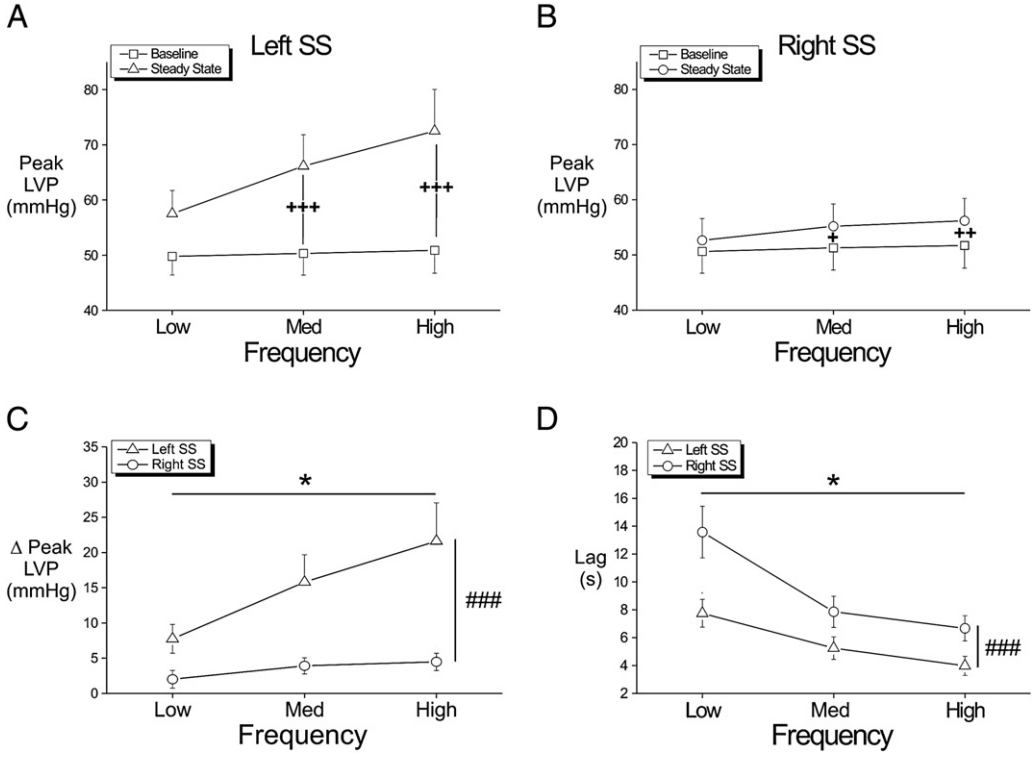

Fig. 5. Effect of unilateral sympathetic stimulation on left ventricular pressure during constant pacing. Mean data illustrating baseline and steady state peak left ventricular pressure (LVP) during left (A) and right (B) sided sympathetic stimulation (SS). The change in LVP from baseline (C) and lag time (time taken to achieve a 1% increase in peak LVP, D). Data represent mean ± SEM, n = 8. Baseline *vs.* steady state: $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$; frequency: $^{*}P<0.05$; left *vs.* right: $^{\#\#\#}P<0.001$.

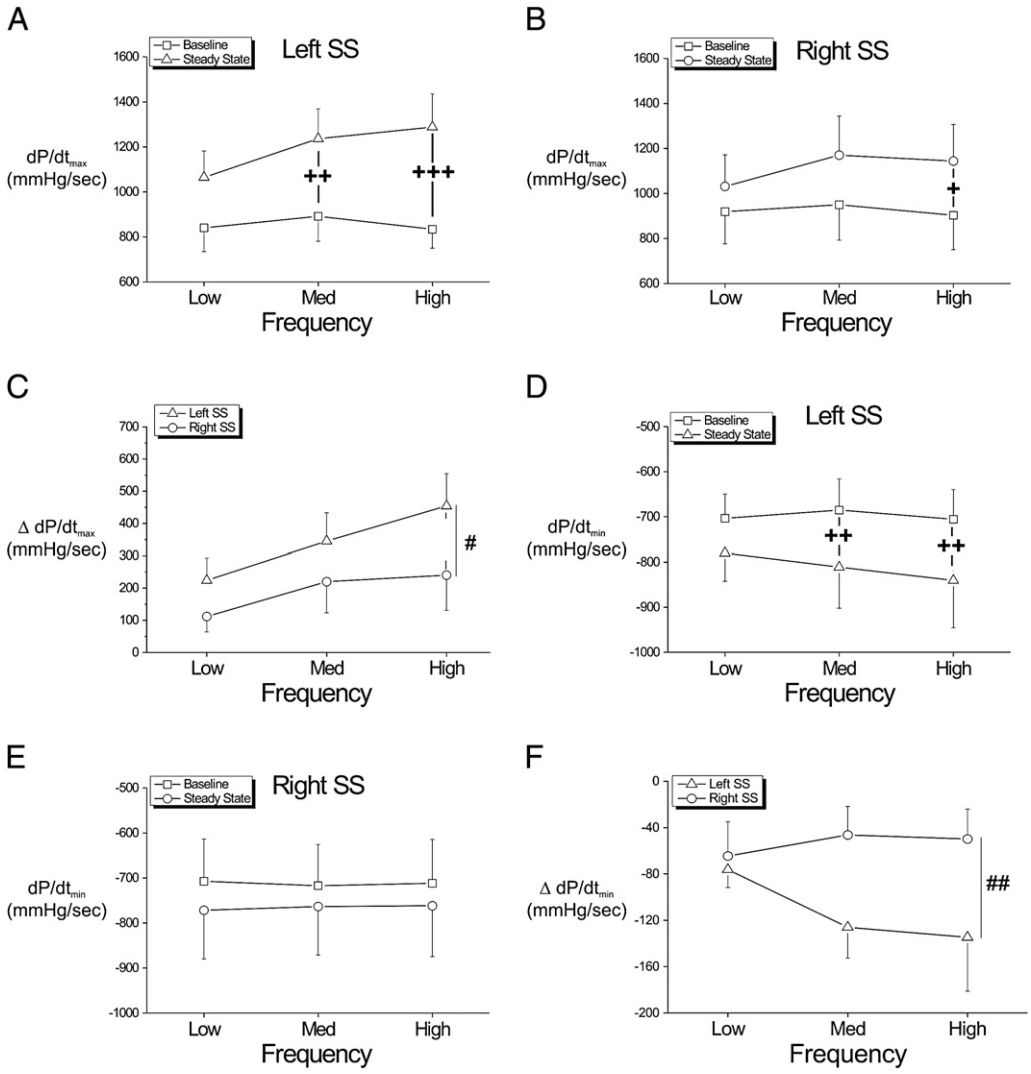

Fig. 6. Effect of unilateral sympathetic stimulation on the rate of change of LV pressure during constant pacing. Mean data illustrating baseline and steady state rates of left ventricular pressure development (dP/dt_{max}) and relaxation (dP/dt_{min}) during left (A and D) and right (B and E) sympathetic stimulation (SS) and the change in dP/dt_{max} (C) and dP/dt_{min} (F) from baseline values. Data represent mean ± SEM, n = 8. Baseline *vs.* steady state: $^{+}P<0.05$, $^{++}P<0.01$; left *vs.* right: $^{\#}P<0.05$, $^{\#\#}P<0.01$.

the AVN whereas nerves arising from the right sympathetic paravertebral chain have a more significant effect on sinus rate. These responses were repeatable and did not show fatigue on repeated testing indicating that the preparation is sustainable over a period of time.

Whilst there is no previous histological or functional information on the relative innervations from the left and right sympathetic chains in the rabbit, unsurprisingly this is in accord with well documented *in vivo* studies in dog and cat (Randall, 1984; Schwartz et al., 1984). In the first of such studies, Randall and Rohse (1956) reported that left stellate stimulation resulted in mainly inotropic enhancement (as assessed through alterations in systolic blood pressure) whilst right stellate stimulation was associated with both inotropic and chronotropic responses *in vivo*. Similarly Randall et al. (1963) reported on the separation of inotropic and chronotropic influences between sympathetic innervations derived from the left and right sympathetic chains. Studies in cats provide similar conclusions in so far as they have shown that the right and left cardiac sympathetics have opposite effects on duration of repolarisation of left ventricular action potentials (Zaza et al., 1991).

4.1. Frequency dependence of sympathetic influence

The responses of the left ventricle to stimulation of the left or right sympathetic chains were frequency dependent probably reflecting temporal summation of the preganglionic action potentials in the stellate ganglion (Birks et al., 1981). The rate of stimulation (2–10 Hz) of the sympathetic chain covers the range of firing observed in preganglionic neurones in the third thoracic segment of anaesthetised rats and cats (Coote, 1988) although even higher rates up to 20 Hz have been recorded following application of glutamate in their vicinity (Coote et al., 1981). In the present study, all the cardiac muscle responses displayed a linear increase to a linear rise in frequency of stimulation of either the right or left sympathetic chains and these responses were preserved over the course of the experiment. However, such artificial stimulation does not mimic the patterns of activity observed in sympathetic preganglionic neurones. Bursts of activity, which is more the norm, could well lead to post-tetanic potentiation, as demonstrated in cardiac nerves of cats following preganglionic stimulation (Birks et al., 1981). Therefore, patterns of sympathetic chain stimulation could possibly give rise to greatly enhanced cardiac effects. The rabbit innervated heart model will provide a highly suitable preparation to study such effects in future studies.

4.2. Regional heterogeneity of sympathetic innervation in the ventricle

There is evidence that sympathetic innervation of ventricular myocardium is heterogeneous with a greater proportion of sympathetic

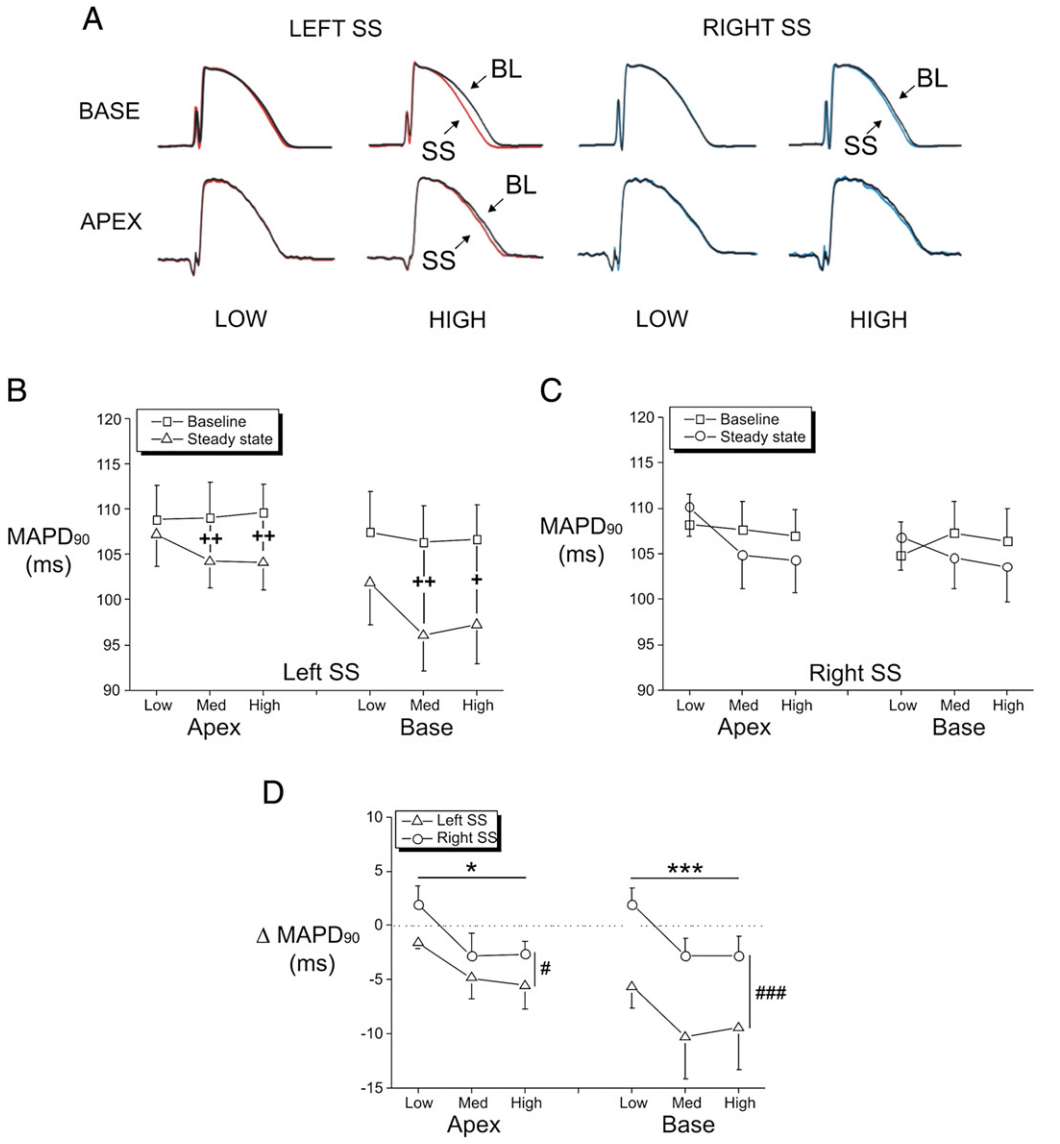

Fig. 7. Effect of unilateral sympathetic stimulation on left ventricular electrophysiological parameters during constant pacing. A) Raw data of the effects of left and right sympathetic stimulation (SS) on apical and basal monophasic action potentials recorded from the left ventricle free wall. B–D) Mean data representing $MAPD_{90}$ at baseline and during SS at apical and basal regions during left SS (B) and right SS (C), and the change in $MAPD_{90}$ during left and right SS relative to baseline (D). BL = baseline. Data represent mean ± SEM, n = 7. Baseline *vs.* steady state: $^{+}P<0.05$, $^{++}P<0.01$; Frequency: $^{*}P<0.05$, $^{***}P<0.001$; left *vs.* right: $^{\#}P<0.05$, $^{\#\#\#}P<0.001$.

nerve fibres at basal than apical regions. We have also shown that both tyrosine hydroxylase and KCNQ1 protein expressions are greater in basal than in apical regions of the LV (Mantravadi et al., 2007). KCNQ1 is a potassium channel protein responsible for the slow delayed rectifier current (IKs) and is thought to mediate the shortening of MAPD associated with β adrenoceptor stimulation. These findings may explain the enhanced effect of left SS on MAPD in basal regions when compared to apical regions in the present study. Previous investigators have described both a gradient of ventricular tissue catecholamine content from the base to the apex (Angelakos, 1965; Petch and Nayler, 1979) and a greater proportion of adrenergic nerve fibres innervating basal regions of the human and canine myocardium (Dahlstrom et al., 1965; Kawano et al., 2003). Basal regions of the myocardium therefore have an increased propensity for MAPD shortening during sympathetic nerve stimulation although the contribution to this phenomenon from the differential expression of sympathetic nerves and/or key ion channels involved in ventricular repolarisation requires further investigation. It is worth noting that tissue from apical regions is more sensitive to adrenergic stimulation by noradrenaline infusion, which has been ascribed to greater β adrenoceptor content in this region (Mori et al., 1993). This mechanism appears to provide some compensation for the sparse sympathetic innervation of this region. This is also supported by our previous study which shows that sympathetic nerve stimulation and isoprenaline produced different effects on the direction of repolarisation in the isolated rabbit heart (Mantravadi et al., 2007).

There is little detailed information on regional differences in the inotropic responses to left and right SS in the rabbit. Our functional data provide information on the net change in contractility across the entire ventricle rather than in specific cardiac regions. In addition MAPs were measured from the anterior portion of the LV free wall and so provide no information on any anterio-posterior variation of response during left or right SS. The existence of differences between anterior and posterior innervation from left and right sympathetic branches is controversial. Yanowitz et al. (1966) described how right stellate ganglionectomy resulted in a prolongation of the functional refractory period at the anterior ventricular surface. The opposite results were noted during left stellate ganglionectomy suggesting that unilateral alterations in sympathetic tone exert influences on the electrophysiological properties of discrete myocardial regions. Regional variations in the contractile response of the myocardium were seen by

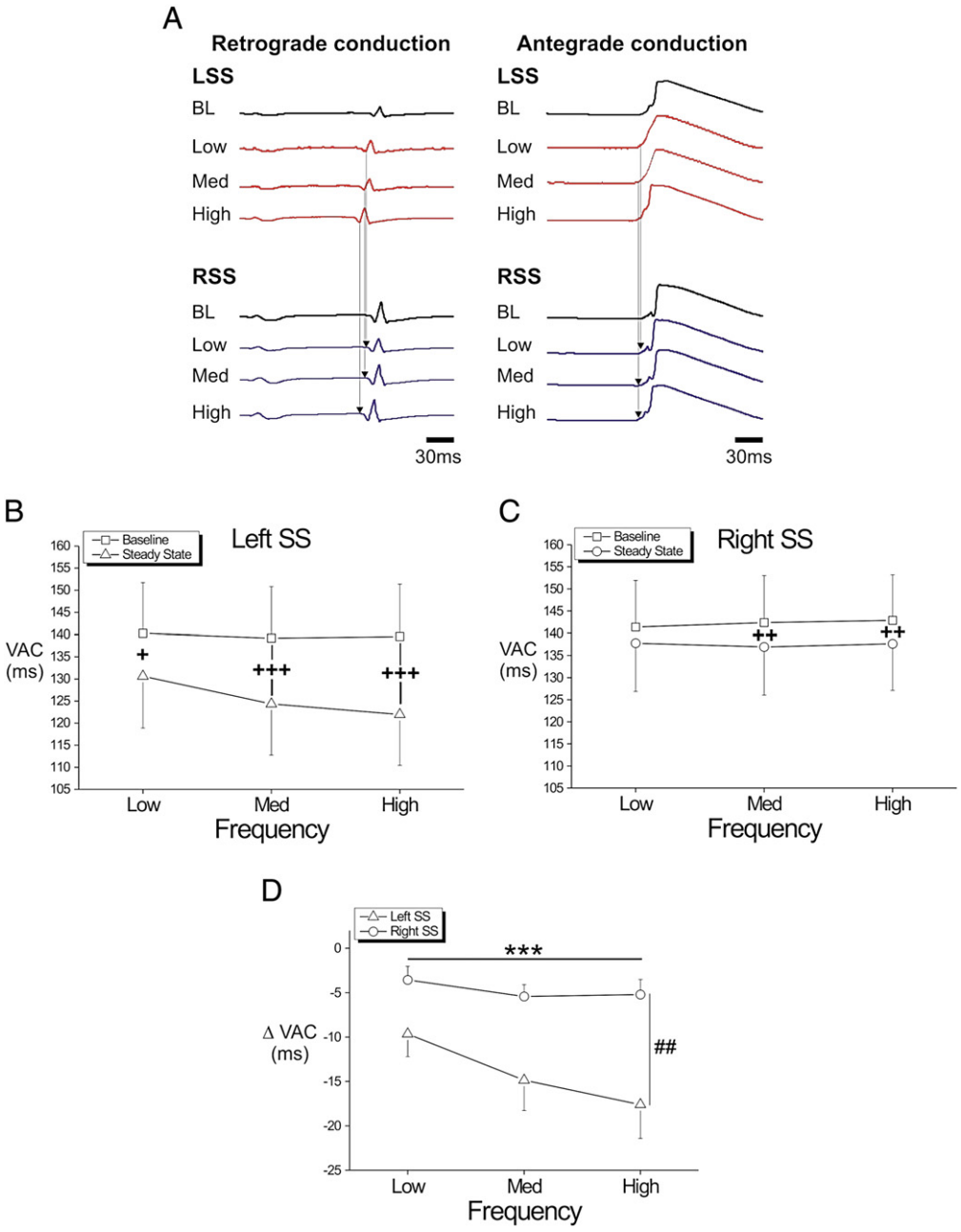

Fig. 8. Effect of unilateral sympathetic stimulation on myocardial dromotropy during constant pacing. A) Raw data illustrating the effects of left and right sympathetic stimulation (SS) on both retrograde (A — left side) and antegrade (A — right side) conduction through the atrio-ventricular node (AVN). Retrograde conduction represents the time taken from a ventricular pacing stimulus to the detection of the atrial electrogram whilst antegrade conduction represents the time taken from an atrial pacing stimulus to the activation upstroke of a monophasic action potential recorded from the left ventricular free wall. The vertical lines demonstrate the activation time of the right atrial electrogram during left SS. Stimulation frequencies are low (2 Hz), medium (5 Hz) and high (10 Hz) frequencies. B–C) Mean data representing ventriculo-atrial conduction (VAC) at baseline and during left (B) and right (C) SS and the difference in VAC from baseline values during left and right SS (C). Data represent mean ± SEM, n = 7. Baseline *vs.* steady state: $^{+}P<0.05$, $^{++}P<0.01$, $^{+++}P<0.001$; frequency: $^{***}P<0.001$; left *vs.* right: $^{\#\#}P<0.01$.

Norris et al. (1977) and lend support to the argument that fibres arising from the left sympathetic chain innervate the posterior surface of the ventricle and that fibres arising from the right sympathetic chain innervate the anterior ventricular surface. In contrast Randall et al. (1968) reported rich overlap of projections arising from the left and right stellate in all regions of the canine ventricle. This is supported by the experiments of Geis and Kaye (1968) combining electrical stimulation of the stellate ganglia and phenol destruction of pre-defined regions of myocardial innervation. These issues may be best addressed by further studies utilising more modern investigative tools such as optical mapping. Such techniques will allow for a detailed understanding of the regional distribution of effects resulting from nerve stimulation.

4.3. Clinical implications

We have previously shown that adrenergic signalling increases the occurrence of triggered ventricular arrhythmias during direct sympathetic nerve stimulation (Ng et al., 2007). Experimental studies, clinical evidence and the findings from the present study suggest that differential innervation plays an important role in the susceptibility of the myocardium to malignant arrhythmias. Stimulation or ablation of either the right or left stellate innervation can have different effects on the ECG (Yanowitz et al., 1966), ventricular refractory periods (Haws and Burgess, 1978; Zaza et al., 1991), VFT (Schwartz et al., 1976a), and incidence of ventricular arrhythmias associated with coronary

occlusion (Schwartz et al., 1976b). Right sided ganglionectomy or left sided sympathetic stimulation (left SS) is associated with an increased susceptibility to sustained ventricular tachycardia, a greater occurrence of delayed afterdepolarisations (DAD) and an augmentation of early-afterdepolarisation (EAD) amplitude whilst right sided sympathetic stimulation (right SS) has less effect on EAD amplitude than left SS (Hanich et al., 1988; Priori et al., 1988). Clinical studies have demonstrated that left cervicothoracic sympathectomy (an adaptation of the stellectomy procedure) significantly reduces the occurrence of adverse cardiac events *e.g.* in patients with congenital long-QT syndrome who are refractory to standard β adrenoceptor antagonists (Schwartz et al., 2004). There is also evidence for the efficacy of left cervicothoracic sympathectomy in the treatment of patients with catecholaminergic polymorphic ventricular tachycardia (Wilde et al., 2008).

The data presented in this manuscript provide evidence that functional consequence from heterogeneous sympathetic innervation exists in an experimental mammals other than the dog suggesting that this may be a common feature in all species. The model also provides an invaluable tool for the study of the mechanisms underlying cardiac arrhythmogenesis, especially ventricular fibrillation. Of particular interest is the potential interaction between the regional differences in sympathetic innervation and regional differences in ion channel distribution (Schram et al., 2002). Together these may have serious implications in understanding arrhythmogenesis not only in cardiac disease states but also in conditions of sympathetic imbalance as a result of extreme emotional stress and central ischaemic stroke.

4.4. Study benefits and limitations

The *in vitro* innervated rabbit heart preparation described in this manuscript provides a number of benefits over traditional *in vivo* approaches allowing for the effects of nerve stimulation to be studied away from the potentially 'confounding' influences of tonic autonomic nervous activity, any stress induced reflex pathways, alterations in circulating hormones and changes in haemodynamic loads. The preparation also provides a novel opportunity for the study of local cardiocardiac reflex loops and the complex interaction between the sympathetic and parasympathetic autonomic systems, whilst the isolated nature of the preparation allows users to control and modify factors such as the coronary perfusion and to investigate the effects of drugs, hormones and other factors (*e.g.* oxygen content). It should be noted that the isolation of this preparation away from 'confounding' physiological factors may represent an over-simplification of the complex process and interactions present in the intact animal and that the patterns of experimental nerve stimulation may not mimic those seen *in vivo*.

4.5. Conclusion

Data presented in this study of the rabbit heart confirm the functional consequences of heterogeneous sympathetic innervation. It extends previous studies on dog and cat, showing a quantitative relationship between stimulation frequency and the effect on ventricular rate, on conduction, on force of contraction and relaxation, and on ventricular monophasic action potential duration. The results demonstrate that unilateral stimulation of sympathetic chains on right and left sides containing cardiac preganglionic nerves can be reliably achieved in an isolated innervated Langendorff rabbit heart preparation. The model presents a novel opportunity to quantitatively examine the direct effects of separate left and right sympathetic nerve stimulation (*i.e.* heterogeneous innervation) on arrhythmogenic mechanisms in the isolated rabbit heart without the influence of confounding neurohumoral, circulatory factors and autonomic reflexes.

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References

Angelakos, E.T., 1965. Regional distribution of catecholamines in the dog heart. Circ. Res. 16, 39–44.
Anzola, J., Rushmer, R.F., 1956. Cardiac responses to sympathetic stimulation. Circ. Res. 4, 302–307.
Ardell, J.L., Randall, W.C., Cannon, W.J., Schmacht, D.C., Tasdemiroglu, E., 1988. Differential sympathetic regulation of automatic, conductile, and contractile tissue in dog heart. Am. J. Physiol. Heart Circ. Physiol. 255, H1050–H1059.
Armour, J.A., 2008. Potential clinical relevance of the 'little brain' on the mammalian heart. Exp. Physiol. 93, 165–176.
Birks, R.I., Laskey, W., Polosa, C., 1981. The effect of burst patterning of preganglionic input on the efficacy of transmission at the cat stellate ganglion. J. Physiol. 318, 531–539.
Boehm, R., Nussbaum, H., 1875. Untersuchungen über den Nervus accelerator cordis der Katze. Naunyn Schmiedebergs Arch. Pharmacol. 4, 255–279.
Brack, K.E., Coote, J.H., Ng, G.A., 2004. Interaction between direct sympathetic and vagus nerve stimulation on heart rate in the isolated rabbit heart. Exp. Physiol. 89, 128–139.
Brack, K.E., Coote, J.H., Ng, G.A., 2006. The effect of direct autonomic nerve stimulation on left ventricular force in the isolated innervated Langendorff perfused rabbit heart. Auton. Neurosci. 124, 69–80.
Brack, K.E., Patel, V.H., Coote, J.H., Ng, G.A., 2007. Nitric oxide mediates the vagal protective effect on ventricular fibrillation via effects on action potential duration restitution in the rabbit heart. J. Physiol. 583, 695–704.
Brack, K.E., Patel, V.H., Mantravadi, R., Coote, J.H., Ng, G.A., 2009. Direct evidence of nitric oxide release from neuronal nitric oxide synthase activation in the left ventricle as a result of cervical vagus nerve stimulation. J. Physiol. 587, 3045–3054.
Brack, K.E., Coote, J.H., Ng, G.A., 2010. Vagus nerve stimulation inhibits the increase in Ca2+ transient and left ventricular force caused by sympathetic nerve stimulation but has no direct effects alone — epicardial Ca^{2+} fluorescence studies using fura-2 AM in the isolated innervated beating rabbit heart. Exp. Physiol. 95, 80–92.
Coote, J.H., Macleod, V.H., Fleetwood-Walker, S., Gilbey, M.P., 1981. The response of individual preganglionic neurones to microelectrophretically applied endogenous amines. Brain Res. 215, 134–145.
Coote, J.H., 1988. The organisation of cardiovascular neurons in the spinal cord. Rev. Physiol. Biochem. Pharmacol. 110, 147–285.
Dahlstrom, A., Fuxe, K., Mya-Tu, M., Zetterstrom, B.E.M., 1965. Observations on adrenergic innervation of dog heart. Am. J. Physiol. 209, 689–692.
Feinberg, H., Katz, L.N., 1958. Effect of catecholamines, l-epinephrine and l-norepinephrine on coronary flow and oxygen metabolism of the myocardium. Am. J. Physiol. 193, 151–156.
Geis, W.P., Kaye, M.P., 1968. Distribution of sympathetic fibers in the left ventricular epicardial plexus of the dog. Circ. Res. 23, 165–170.
Hanich, R., Levine, J., Spear, J., Moore, E., 1988. Autonomic modulation of ventricular arrhythmia in cesium chloride-induced long QT syndrome. Circulation 77, 1149–1161.
Haws, C.W., Burgess, M.J., 1978. Effects of bilateral and unilateral stellate stimulation on canine ventricular refractory periods at sites of overlapping innervation. Circ. Res. 42, 195–198.
Hopkins, D.A., Armour, J.A., 1984. Localization of sympathetic postganglionic and parasympathetic preganglionic neurons which innervate different regions of the dog heart. J. Comp. Neurol. 229, 186–198.
Hopkins, D.A., Armour, J.A., 1989. Ganglionic distribution of afferent neurons innervating the canine heart and cardiopulmonary nerves. J. Auton. Nerv. Syst. 26, 213–222.
Kawano, H., Okada, R., Yano, K., 2003. Histological study on the distribution of autonomic nerves in the human heart. Hear. Vessel. 18, 32–39.
Mantravadi, R., Gabris, B., Liu, T., Choi, B.-R., de Groat, W.C., Ng, G.A., Salama, G., 2007. Autonomic nerve stimulation reverses ventricular repolarization sequence in rabbit hearts. Circ. Res. 100, e72–e80.
Mori, H., Ishikawa, S., Kojima, S., Hayashi, J., Watanabe, Y., Hoffman, J.I.E., Okino, H., 1993. Increased responsiveness of left ventricular apical myocardium to adrenergic stimuli. Cardiovasc. Res. 27, 192–198.
Ng, G., Brack, K., Coote, J., 2001. Effects of direct sympathetic and vagus nerve stimulation on the physiology of the whole heart—a novel model of isolated Langendorff perfused rabbit heart with intact dual autonomic innervation. Exp. Physiol. 86, 319–329.
Ng, G.A., Brack, K.E., Patel, V.H., Coote, J.H., 2007. Autonomic modulation of electrical restitution, alternans and ventricular fibrillation initiation in the isolated heart. Cardiovasc. Res. 73, 750–760.
Ng, G.A., Mantravadi, R., Walker, W.H., Ortin, W.G., Choi, B.-R., de Groat, W., Salama, G., 2009. Sympathetic nerve stimulation produces spatial heterogeneities of action potential restitution. Hear. Rhythm. 6, 696–706.
Norris, J.E., Lippincott, D., Wurster, R.D., 1977. Responses of canine endocardium to stimulation of the upper thoracic roots. Am. J. Physiol. Heart Circ. Physiol. 233, H655–H659.

Petch, M.C., Nayler, W.G., 1979. Concentration of catecholamines in human cardiac muscle. Br. Heart J. 41, 340–344.
Priori, S., Mantica, M., Schwartz, P., 1988. Delayed afterdepolarizations elicited in vivo by left stellate ganglion stimulation. Circulation 78, 178–185.
Randall, W.C., Rohse, W.G., 1956. The augmentor action of the sympathetic cardiac nerves. Circ. Res. 4, 470–475.
Randall, W.C., Priola, D.V., Ulmer, R.H., 1963. A functional study of distribution of cardiac sympathetic nerves. Am. J. Physiol. 205, 1227–1231.
Randall, W.C., Szentivanyi, M., Pace, J.B., Wechsler, J.S., Kaye, M.P., 1968. Patterns of sympathetic nerve projections onto the canine heart. Circ. Res. 22, 315–323.
Randall, W.C., 1984. Selective autonomic innervation of the heart. In: Randall, W.C. (Ed.), Nervous Control of the Heart. Oxford University Press, United Kingdom. ISBN: 0195033906, pp. 46–47.
Rona, G., Chappel, C.I., Balazs, T., Gaudry, R., 1959. An infarct-like myocardial lesion and other toxic manifestations produced by isoproterenol in the rat. AMA Arch. Pathol. 67, 443–455.
Rona, G., 1985. Catecholamine cardiotoxicity. J. Mol. Cell. Cardiol. 17, 291–306.
Schram, G., Pourrier, M., Melnyk, P., Nattel, S., 2002. Differential distribution of cardiac ion channel expression as a basis for regional specialization in electrical function. Circ. Res. 90, 939–950.
Schwartz, P.J., Snebold, N.G., Brown, A.M., 1976a. Effects of unilateral cardiac sympathetic denervation on ventricular fibrillation threshold. Am. J. Cardiol. 37, 1034–1040.
Schwartz, P.J., Stone, H.L., Brown, A.M., 1976b. Effects of unilateral stellate ganglion blockade on the arrhythmias associated with coronary occlusion. Am. Heart J. 92, 589–599.
Schwartz, P.J., Billman, G.E., Stone, H.L., 1984. Autonomic mechanism in ventricular fibrillation induced by myocardial ischemia during exercise in dogs with healed myocardial infarction. An experimental preparation for sudden cardiac death. Circulation 69, 70–800.
Schwartz, P.J., Priori, S.G., Cerrone, M., Spazzolini, C., Odero, A., Napolitano, C., Bloise, R., De Ferrari, G.M., Klersy, C., Moss, A.J., Zareba, W., Robinson, J.L., Hall, W.J., Brink, P. A., Toivonen, L., Epstein, A.E., Li, C., Hu, D., 2004. Left cardiac sympathetic denervation in the management of high-risk patients affected by the long-QT Syndrome. Circulation 109, 1826–1833.
Sonnenblick, E.H., Siegel, J.H., Sarnoff, S.J., 1963. Ventricular distensibility and pressure–volume curve during sympathetic stimulation. Am. J. Physiol. 204, 1–4.
Spear, J.F., Moore, E.N., 1973. Influence of brief vagal and stellate nerve stimulation on pacemaker activity and conduction within the atrioventricular conduction system of the dog. Circ. Res. 32, 27–41.
Vassalle, M., Levine, M.J., Stuckey, J.H., 1968. On the sympathetic control of ventricular automaticity: the effects of stellate ganglion stimulation. Circ. Res. 23, 249–258.
Wilde, A.A.M., Bhuiyan, Z.A., Crotti, L., Facchini, M., De Ferrari, G.M., Paul, T., Ferrandi, C., Koolbergen, D.R., Odero, A., Schwartz, P.J., 2008. Left cardiac sympathetic denervation for catecholaminergic polymorphic ventricular tachycardia. N. Engl. J. Med. 358, 2024–2029.
Yanowitz, F., Preston, J.B., Abildskov, J.A., 1966. Functional distribution of right and left stellate innervation to the ventricles: production of neurogenic electrocardiograph changes by unilateral alteration of sympathetic tone. Circ. Res. 18, 416–428.
Zaza, A., Malfatto, G., Schwartz, P.J., 1991. Sympathetic modulation of the relation between ventricular repolarization and cycle length. Circ. Res. 68, 1191–1203.