Title

Chronic spinal cord stimulation modifies intrinsic cardiac synaptic efficacy in the suppression of atrial fibrillation

Journal

Autonomic Neuroscience, 186(C)

ISSN

1566-0702

Authors

Ardell, Jeffrey L
Cardinal, René
Beaumont, Eric
et al.

Publication Date

2014-12-01

DOI

10.1016/j.autneu.2014.09.017

Peer reviewed

HHS Public Access

Author manuscript

Auton Neurosci. Author manuscript; available in PMC 2015 December 01.

Published in final edited form as:

Auton Neurosci. 2014 December ; 186: 38–44. doi:10.1016/j.autneu.2014.09.017.

Chronic spinal cord stimulation modifies intrinsic cardiac synaptic efficacy in the suppression of atrial fibrillation

Jeffrey L. Ardell[1,*], **René Cardinal**[2,*], **Eric Beaumont**[3], **Michel Vermeulen**[2], **Frank M. Smith**[4], and **J. Andrew Armour**[1]

[1]Department of Medicine, UCLA Health System, Los Angeles, CA

[2]Department of Pharmacology, Faculté de médecine, Université de Montréal and Centre de recherche, Hôpital du Sacré-Cœur de Montreal, Montréal, Quebec

[3]Department of Biomedical Sciences, Quillen College of Medicine, East Tennessee State University, Johnson City, Tennessee

[4]Department of Medical Neuroscience, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia

Abstract

We sought to determine whether spinal cord stimulation (SCS) therapy, when applied chronically to canines, imparts long-lasting cardio-protective effects on neurogenic atrial tachyarrhythmia induction and, if so, whether its effects can be attributable to i) changes in intrinsic cardiac (IC) neuronal transmembrane properties vs ii) modification of their interneuronal stochastic interactivity that initiates such pathology. Data derived from canines subjected to long-term SCS [(group 1 studied after 3–4 weeks SCS; n=5) (group 2: studied 5 weeks SCS; n=11)] were compared to data derived from 10 control animals (including 4 sham SCS electrode implantations). During terminal studies conducted under anesthesia, chronotropic and inotropic responses to vagal nerve or stellate ganglion stimulation were similar in all 3 groups. Chronic SCS suppressed atrial tachyarrhythmia induction evoked by mediastinal nerve stimulation. When induced, arrhythmia durations were shortened (controls: median of 27s; SCS 3–4 weeks: median of 16s; SCS 5 weeks: median of 7s). Phasic and accommodating right atrial neuronal somata displayed similar passive and active membrane properties *in vitro*, whether derived from sham or either chronic SCS groups. Synaptic efficacy was differentially enhanced in accommodating (not phasic) IC neurons by chronic SCS. Taken together these data indicate that chronic SCS therapy

Correspondence: Jeffrey L. Ardell, Ph.D., Department of Medicine (Cardiology), UCLA Arrhythmia Center and UCLA Neurocardiology Research Center of Excellence, UCLA Health System, 47-129 CHS, Los Angeles, CA 90095, jardell@mednet.ucla.edu, 310-825-0417.

*both authors contributed equally.

Author contributions: JLA, RC and JAA contributed to the conception and design of experiments. EB, MV, FS performed experiments and analyzed the data. JLA, RC, EB and JAA drafted, edited and revised the manuscript. All authors approved the final version of the manuscript.

Conflict of interest: None

modifies IC neuronal stochastic inter-connectivity in atrial fibrillation suppression by altering synaptic function without directly targeting the transmembrane properties of individual IC neuronal somata.

Keywords

atrial tachyarrhythmia; intrinsic cardiac neurons; spinal cord stimulation; neuromodulation; heart

Introduction

Clinical evidence indicates that symptoms associated with chronic refractory angina of cardiac origin can be alleviated by delivering high frequency, low intensity electrical stimuli to the dorsal aspect of the thoracic spinal cord - spinal cord stimulation (SCS) therapy (Eliasson *et al.*, 1996; Mannheimer *et al.*, 2002). It is known that the myocardium state is transduced by cardiac sensory neurites that are associated with somata nexus points throughout the cardiac nervous system, including intrathoracic ganglia and central loci (Armour & Kember, 2004; Longhurst *et al.*, 2001; Tjen-A-Looi *et al.*, 1997; Zucker *et al.*, 2012). All of these populations directly and indirectly influence the behavior of intrinsic cardiac neurons, including intrinsic cardiac local circuit neurons (Ardell, 2004; Armour, 2008).

Excessive activation of selective neuronal inputs to the intrinsic cardiac nervous system (ICNS) – for instance by select mediastinal nerve stimulation – consistently initiates atrial tachyarrhythmias in the canine model (Armour *et al.*, 2005; Cardinal *et al.*, 2010). It is also known that SCS, when applied acutely, modifies the behavior of select populations of intrinsic cardiac neurons, in particular its local circuit neuronal population (Armour *et al.*, 2002; Foreman *et al.*, 2000), to obtund atrial tachyarrhythmias of neuronal origin (Cardinal *et al.*, 2006). Recent evidence indicates that the efficacy of acute SCS may reside primarily in its capacity to stabilize ICNS local circuit neurons in the presence of such excessive and heterogeneous neuronal inputs (Gibbons *et al.*, 2012).

While the cardiac nervous system is optimized to respond to every day stressors (heat, exercise, emotion, orthostatic), it can be critically disrupted by cardiac pathology such as myocardial ischemia, myocardial infarction, heart failure and chronic arrhythmias (Ajijola *et al.*, 2013; Nakahara *et al.*, 2010; Kember *et al.*, 2013; Zucker *et al.*, 2012; Dell'Italia, 2011). In contradistinction to global and non-specific effects of pharmacological management of such pathologies (Brunton *et al.*, 2010), chronic neuromodulation based approaches offer the opportunity to target relevant elements of the cardiac nervous system and, as a consequence, influence the cardiomyocytes they regulate (Lopshire & Zipes, 2012; Liu *et al.*, 2012; Schwartz, 2012). What remains to be determined is the short versus long term effects of such therapy in the context of specific cardiac pathologies.

It is known that long-term application of SCS prevents the development of a tachypacing-induced atrial fibrillation (Bernstein *et al.*, 2012), an effect that has been proposed to depend on electrophysiological remodeling of cardiac myocyte ionic channels (Lopshire *et al.*, 2009). However, based on our prior work (Beaumont *et al.*, 2013; Gibbons *et al.*, 2012;

Cardinal *et al.*, 2006), it is likely that the intrinsic cardiac nervous system, especially its local circuit neurons (Gibbons *et al.*, 2012), represent a major target for this reduced arrhythmia potential. It remains to be established whether chronic SCS therapy imparts long-term effects on the intrinsic cardiac nervous system. It also remains to be established whether chronic SCS therapy: 1) targets the function of individual neurons within the intrinsic cardiac nervous system such that their membrane excitability to neuronal inputs becomes modified (Cardinal *et al.*, 2004) vs 2) suppressing the stochastic network hyper-interactivity that occurs among populations of intrinsic cardiac local circuit neurons in the induction of atrial arrhythmias (Beaumont *et al.*, 2013) vs 3) a combination of both processes.

In order to understand these core issues, chronic SCS (3–4 weeks vs 5 weeks) was applied in normal canines to determine the impact of this therapy on the capacity of the intrinsic cardiac nervous system to initiate atrial tachyarrhythmias. By these means, we found that the primary target of chronic SCS therapy in atrial arrhythmia treatment resides in its capacity to regulate intrinsic cardiac neuronal network excitability, rather than solely targeting and remodeling the function of individual intrinsic cardiac neurons. If such a thesis is sustained it implies that suppression of hyper-excited, stochastic interactions within this target organ's nervous system that lead to pathology represents a potential target for anti-arrhythmia therapy.

Materials and methods

Animals

Experiments were approved by the Animal Research Ethics Committee of the Sacré-Coeur Hospital Research Centre and were in accordance with the Guide for the Care and Use of Laboratory Animals, Eighth Edition, National Academy Press, Washington DC, 2010. Three groups of canines were studied: *i)* 5 canines were subjected to continuous spinal cord stimulation for 21–27 days (3–4 weeks); *ii)* 11 canines were subjected to continuous SCS for 33–35 days (5 weeks); and *iii)* 10 control animals were studied that included 4 canines in which a SCS electrode and neurostimulator were implanted (without being activated) for 5 weeks (sham SCS) and 6 control animals without electrode implantation, all kept in the same housing environment.

Spinal cord stimulator implantation and SCS implementation

Under isoflurane (2%) anaesthesia and sterile conditions, animals were placed in the prone position; a small incision was made on the dorsal surface (T4–T6 level) and the epidural space of the mid-thoracic spinal column penetrated percutaneously with a Touhy needle, using fluoroscopic guidance and loss-of-resistance technique. Then an octopolar electrode (Octrode™ Model 3086, St. Jude Medical, Plano TX) was introduced *via* this cannula into the epidural space and its tip advanced to the T1 spinal level, slightly to the left of midline. The rostral and caudal poles selected for subsequent use (spacing of 52 mm) were placed at the T1 and T4 levels. These were connected to a neurostimulator (EonC™ Model 3688, St. Jude Medical) generating 50Hz, 0.2-ms duration pulses. An intensity setting of 90% of motor threshold (contraction of proximal forepaw, shoulder, and thoracic trunk musculature)

was determined. After fixing the electrodes in place, the lead was tunneled to a subcutaneous pouch created on the animal flank, both incisions were closed in layers and the animal recovered from surgery. The pulse generator was inactive for the duration of the recovery period (~1 week). Following recovery from implant, SCS was applied for 3–4 (n = 5 dogs) or 5 (n = 11 dogs) weeks.

Terminal *in situ* studies

Anesthesia was reapplied with Na thiopental (25 mg/kg i.v.). Animals were intubated and ventilation maintained under positive-pressure. Through a midline neck incision each vagosympathetic trunk was exposed and sectioned cephalad to the site of electrical stimulation electrode application. A bilateral thoracotomy was then performed and the pericardium was incised to expose the heart. Both stellate ganglia were exposed and their central connections severed. Left ventricular pressure (model SPC-350 electronic pressure sensors, Millar, Houston, TX) and a lead II ECG were recorded on a rectilinear pen recorder (Nihon Kohden, Tokyo, Japan). All hemodynamic data were digitized (Cambridge Electronic Design power 1401 acquisition system with Spike 2 software) for subsequent off-line analysis.

After completion of these surgical manipulations, the anesthesia was changed to α-chloralose (25–50 mg/kg iv bolus supplemented with 25 mg/kg iv as required). To establish baseline levels for extrinsic efferent control of regional cardiac function, the right and left vagosympathetic trunks (frequency: 3,5,10 and 20 Hz) and stellate ganglia (4 Hz) were stimulated individually using bipolar electrodes connected to a battery-driven current source controlled by programmable stimulator (2 ms pulse duration; 3× threshold intensity). To evaluate the atrial arrhythmogenic substrate, mediastinal nerves were first visualized coursing over the ventral or ventrolateral surface of the superior vena cava within the pericardial reflection. Trains of 5 electrical stimuli (2 ms pulse width; 5 ms inter-pulse interval) were then applied via bipolar electrodes (1.5mm apart) to identified nerves once *per* cardiac cycle during the refractory period of neighboring atrial tissues (*i.e.* ~30 ms after excitation of a reference bipolar atrial electrogram). This procedure was employed to avoided direct atrial muscle capture *via* the bipolar electrodes. The electrodes, mounted on a hand held probe, were connected to a battery-driven constant current stimulus isolator (model A385, World Precision Instruments, Inc., Sarasota FL). The current delivered via this probe was controlled by a programmable stimulator triggered by the reference atrial electrogram. With this technique, atrial arrhythmias can be reproducibility activated over hours and the efficacy of specific neuromodulation therapies evaluated (Armour *et al.*, 2005; Gibbons *et al.*, 2012; Richer *et al.*, 2008).

Three mediastinal nerve sites were identified in each animal. The stimulus intensity applied to identified mediastinal nerves via the bipolar electrodes was 1 mA at first in order to determine if stimuli applied to an identified nerve could elicit sinus bradycardia that was rapidly followed by atrial tachyarrhythmia induction, as reported previously (Armour *et al.*, 2005; Beaumont *et al.*, 2013). The stimulus current was stopped immediately upon tachyarrhythmia induction. After a few minutes of recovery, stimulus application was repeated at 2 mA intensity and a third trial was later performed at 5 mA.

Terminal *in vitro* studies

In 4 control (sham SCS) and 8 of the SCS 5 weeks animals (at the end of the experimental period), in the presence of general anesthesia the heart was excised. The part of the right atrial free wall which contains the right atrial ganglionated plexus was removed quickly from the heart and placed in a dish containing cold (4°C) Tyrode's solution (composition in mM: NaCl 120, $NaHCO_3$ 25, NaH_2PO_4 1, KCl 5, $MgCl_2$ 2, $CaCl_2$ 2.5, D-glucose 11; pH 7.4). The myocardium surrounding the fat containing the right atrial ganglionated plexus was trimmed away. The remaining tissue was pinned with the endocardial surface placed on the silicone-rubber covering the bottom of a recording chamber. The recording chamber (volume: 5 ml) was continuously superfused (5–8 ml min^{-1}) by gravity from a reservoir filled with Tyrode's solution saturated with a gas mixture of 95% O_2 and 5% CO_2 to ensure adequate tissue oxygenation and pH (7.4). The temperature of this solution was maintained at 34°C.

The preparation was epi-illuminated and viewed through a stereo microscope. The epicardial sheath was removed and the underlying plexus of ganglia and nerves identified. Ganglia, usually located at the junctions of two or more nerves, were mechanically stabilized for microelectrode impalement with the aid of a small metal platform inserted under the ganglion. Microelectrodes, derived from borosilicate glass tubing (0.5 mm ID, 1.0 mm OD with internal filament; type BF-100-50-10, Sutter Instruments, Novato, CA), were pulled to obtain a resistance range of 20–50 MΩ when filled with 3M KCl. These electrodes were advanced via a mechanical manipulator (MX-4, Narishige, Japan) into the ganglion under study to impale neuronal somata. The microelectrode was coupled to an intracellular amplifier (model 1600, A-M Systems, Everett, WA) operated in current clamp mode. After recording from each cell, the microelectrode was withdrawn from the ganglion into the bath and the null potential re-checked.

Successful impalement was signalled by a sudden deflection of the electrode potential to a stable negative value. Criteria for accepting a cell for study were as follows: stable resting membrane potential more negative than −40 mV; action potentials (AP) with peak potential overshoot evoked reproducibly by current pulses delivered *via* the recording electrode. Plexus nerves that connected to the studied ganglion were stimulated extracellularly *via* bipolar silver electrodes coupled to a constant current or voltage stimulus isolation unit (model 2200, A–M Systems) controlled by Spike 2.0 software.

- **i.** *Membrane potential and AP properties*. Once resting membrane potentials had stabilized, 5 ms-depolarizing current pulses were applied intracellularly at 1 s intervals with increasing current (0.1–1nA in 0.1 nA steps) until threshold of AP induction was identified. AP indices of amplitude and duration were determined from that ensuing spike. After hyperpolarization duration was measured at 50% recovery from maximum hyperpolarization potential.
- **ii.** *Membrane input resistance:* Current pulses (1 s duration) of increasing intensity (0.1 to −0.6 nA) were injected intracellularly in 0.1 nA steps. Resultant voltage displacements (measured at 800 ms) were plotted as a function of current intensity,

and the slope of this relationship was used to determine membrane input resistance of individual neurons.

iii. *Repetitive firing properties and membrane excitability:* These indices were evaluated by applying 1 second depolarizing current pulses intracellularly at 5 s intervals, with increasing current (0.1 to 1nA in 0.1 nA) steps.

iv. *Membrane responses to stimulation of plexus nerve inputs*. Single or repetitive pulses (0.5 ms duration; 100 μA - 5 mA) were then applied extracellularly to one or more nerves connected to the ganglion containing the impaled neuron. Single-pulse stimuli applied at an intensity that exceeded the activation threshold for presynaptic axons frequently elicited a neuronal excitatory postsynaptic potential (EPSP) which upon reaching threshold usually elicited an action potential. To test synaptic transfer function, these nerves were then stimulated (2× threshold intensity for AP generation) with trains of electrical pulses (0.5 ms duration) applied at 20s intervals at increasing frequencies (5 pulse-trains at 0.2 and 0.5 Hz; 10 pulse-trains at 1 and 2 Hz; 25 pulse-trains at 5 Hz; 50 pulse-trains at 10 Hz and 100 pulse-trains at 20 Hz). Transmembrane potentials and applied stimulus waveforms were analyzed on line (via an oscilloscope), as well as being stored on the hard drive of a computer (via Spike 2 software) for later analysis.

Data analysis

***In situ* studies—**Heart rate (HR) changes from basal values during right or left vagus stimulation were analyzed by ANOVA. HR and LV dP/dt_{max} data obtained during right or left stellate ganglion stimulation were also compared among groups. Cardiac cycle length was measured for successive atrial cycles during mediastinal nerve stimulation to compare the incidence of atrial tachyarrhythmias induced in response to mediastinal nerve stimulation at 1, 2, and 5 mA stimulation intensities in control and SCS (3–4 weeks or 5 weeks) treated groups. These data were subjected to Freeman-Tukey transformation and ANOVA (within and among groups). As atrial tachyarrhythmia durations did not follow a normal distribution, the durations of tachyarrhythmia episodes induced among control and SCS (3–4 weeks or 5 week) groups were compared employing non-parametric Kruskal-Wallis statistics. Differences were considered as statistically significant when $p \leq 0.05$.

***In vitro* studies—**Intrinsic cardiac neurons derived from normal or SCS treated animals were classified as ‘phasic’ or ‘accommodating’ on the basis of their firing patterns elicited during intracellularly applied current steps of 1s duration. Transmembrane membrane and action potential (AP) data derived from individual neuronal somata of control (sham SCS) and chronic SCS (5 week) animals were compared. *t*-test comparisons were utilized for data derived from the same neuron types among groups. Synaptic efficacy (% output/input) for individual IC neurons subsequent to presynaptic activation (2 to 20 Hz) were compared among neurons derived from control vs SCS (5 weeks SCS) groups using ANOVA for repeated measures.

Results

In vivo studies

Vagosympathetic complex or stellate ganglion stimulation (Figure 1)—Heart rate decrements in response to right or left vagus nerve stimulation were similar in control and chronic SCS treated animals. Heart rate, LV chamber pressure and dP/dt_{max} enhancement responses to right or left stellate ganglion stimulation were also similar among groups.

Mediastinal nerve stimulation induction of atrial tachyarrhythmias—In the anesthetized control animals (n=10), 4 of which had chronically implanted spinal cord electrodes that were not activated, focal electrical stimuli delivered individually to multiple mediastinal nerves reproducibly evoked atrial tachyarrhythmias. Figure 2A illustrates one such example in which, in response to three consecutive right-sided mediastinal nerve stimulations, there was a bradycardia that rapidly transitioned into atrial tachyarrhythmia that lasted for 58s before spontaneous reversion to sinus rhythm. On average it required 5.4±3.7 trains of electrical stimuli to evoke tachyarrhythmias; tachyarrhythmia induction was consistently preceded by a transient cycle length prolongation (from 377±74 to 478±112 ms). Overall, there was a positive correlation to stimulus intensity (Figure 3A) and an average duration of the induced tachyarrhythmias of 27s (Figure 3B).

Effects of chronic SCS on the atrial arrhythmogenic substrate—After 3–4 weeks of SCS, 40% of nerve sites tested at 2mA elicited atrial tachyarrhythmias - with the average duration of the atrial tachyarrhythmias decreased by ~40% (27s to 16s) (Figure 3). After 5 weeks of continuous SCS, 6% of identified sites induced atrial tachyarrhythmias at the 2mA levels and the duration of the tachyarrhythmias in residual active sites was reduced by 74% of control (27s to 7s). Among the animals treated with 5 weeks of SCS, atrial tachyarrhythmias could not be induced in 5 of the 11 animals, even when repetitive stimuli were delivered at stimulus intensities of 5 mA (Figure 2B). As summarized in Figure 3A, the relationship between cumulative tachyarrhythmia incidence and stimulus strength was altered in chronic SCS treated animals ($p<0.001$), being significantly lower than control in the 5 weeks SCS group ($p=0.014$). Latency to induction of AF in response to mediastinal nerve stimulation trended towards higher levels with chronic SCS (2.2 sec sham, 2.7 sec 3–4 week SCS and 4.4 sec at 5 week SCS); average AF cycle length did not change (sham 119±14 ms; 117±16ms SCS 3–4 weeks; 119±13ms SCS 5 weeks). Following *in vivo* assessment of the arrhythmia potential, the right atrial ganglionated plexus was harvested from a subset of these animals so that their individual passive and active membrane properties could be assessed *in vitro*.

In vitro studies

Passive and active soma membrane properties—31 intrinsic cardiac (IC) neurons derived from the right atrial ganglionic plexus of 4 control hearts and 76 IC neurons derived from 8 hearts with 5 weeks SCS were studied. Neurons were classified according to their phasic or accommodating behavior, depending on whether action potential (AP) discharge ceased within or continued after the first 100 ms of a 1 s-intracellular applied depolarizing

current pulses, respectively. Phasic neurons typically discharged 1–2 APs during the first 100 ms of intracellular depolarization (Fig 4A). There was no distinguishable difference in the discharge patterns of phasic neurons derived from control vs chronic SCS treated animals in response to step changes in depolarizing current (Fig 4B). Table 1 summarizes the membrane properties of each subtype of neuron with and without chronic SCS. While within group comparisons substantiate the expected differences in threshold current and input resistance between phasic and accommodating IC neurons, chronic SCS did not alter any primary components of evoked discharge or recovery potential for either functional type of IC neuron. While there was a trend towards decreased input resistance between SCS and control for both neuron sub-types, this did not reach significance.

Postsynaptic responses to nerve stimulation—Figure 5 summarizes the effects of stimulating plexus nerves closely adjacent to recorded IC neurons with extracellular currents (at frequencies from 2–20 Hz) and their orthodromic responses characterized with responses sub-grouped based on their classification as phasic or accommodating cells (see Figure 4). Across all stimulation frequencies, IC synaptic efficacy (defined as percent action potential output/stimulus pulse input) between control and SCS treatment groups was 82.8 ± 4.6% versus 83.4 ± 7.0% (p=0.85) for phasic neurons and 87.0 ± 5.7% versus 94.1 ± 5.7% (p=0.04) for accommodating neurons. Within treatment group comparisons indicated that while there was no significant difference between average synaptic efficacy in control states between phasic and accommodating neurons, with chronic SCS treatment the accommodating neurons became differentially enhanced (83.4 ± 7.0% phasic versus 94.1 ± 5.7% accommodating; p = 0.008).

Discussion

The main findings derived from this study indicate that long-term SCS therapy imparts sustained cardioprotection with regard to the induction of atrial tachyarrhythmias in response to excessive, asymmetric activation of the intrinsic cardiac nervous system (ICNS) and that the efficacy of that arrhythmia stabilization increases with duration of treatment. Moreover, the chronic effects that SCS imparts to the intrinsic cardiac nervous system are not primarily associated with any modification of the electrical properties of individual ICNS neuronal somata. Rather, they reside primarily in the capacity of chronic SCS therapy to influence how the entire intrinsic cardiac nervous system synaptically transduces excessive neuronal inputs in the induction of atrial fibrillation.

It is known that acute application of SCS obtunds the effects of excessive ICN activation to suppress initiation of neurally induced atrial tachyarrhythmias (Cardinal *et al.*, 2006; Gibbons *et al.*, 2012). We have further shown that acute SCS exerts its anti-arrhythmic effects primarily by targeting/stabilizing the local circuit neurons (processing elements) of the intrinsic cardiac nervous system (Gibbons *et al.*, 2012) rather than the direct pathways mediated by primary sympathetic and parasympathetic efferent inputs to the heart (Beaumont *et al.*, 2013). Furthermore, it is known that such acute neuromodulation effects have a neural memory of at least 1 hour (Armour *et al.*, 2002). While it has been shown that long-term application of SCS prevents the development of a tachypacing-induced atrial fibrillation (Bernstein *et al.*, 2012) and that chronic SCS reduces the potential for ventricular

arrhythmias post-infarct progressing into heart failure (Lopshire *et al.*, 2009), little is known regarding the impact of chronic SCS on the peripheral aspects of the cardiac nervous system.

A primary finding of this study is that the efficacy for arrhythmia control in response to induced-neural imbalances within the intrinsic cardiac nervous system appears to be time-dependent and, as such, was enhanced with chronic SCS. While the tachyarrhythmia induction in response to mediastinal nerve stimulation was blunted significantly in animals subjected to 3–4 weeks of SCS therapy, these anti-arrhythmic effects were even greater after 5 weeks of SCS. Furthermore, even for those residual neurally induced tachyarrhythmias their duration was reduced by 40% following weeks 3–4 weeks of SCS and by 74% after 5 weeks of SCS. The *in vivo* data further indicate that this reduction was not associated with functional modification of the primary efferent neuronal inputs to intrinsic cardiac neurons (c.f. Figure 1), but rather to how the intrinsic cardiac neural network responds as a collective to the imposed stress of excessive, asymmetric activation.

It has been postulated that spinal cord inputs to the ICNS, when modified by acute SCS, stabilizes the intrinsic cardiac nervous system (Foreman *et al.*, 2000; Gibbons *et al.*, 2012) such that excessive, asymmetric mediastinal nerve inputs to the ICNS have a reduced capacity to initiate atrial tachyarrhythmias (Cardinal *et al.*, 2006; Gibbons *et al.*, 2012). Such SCS neuromodulation-induced network stabilization in fact mitigates excessive and heterogeneous efferent outputs to the atrial cardiomyocytes, an effect that by itself would be electrically destabilizing (Chen & Tan, 2007).

The evolution of the anti-arrhythmogenic efficacy of chronic SCS could reflect changes induced in i) neuronal soma, ii) synaptic function and/or iii) local neuronal network interactions. Evidence presented herein indicates that changes in soma passive and active membrane properties were not a primary adaptive response elicited by chronic SCS. Rather, our data demonstrate that reorganization of synaptic processing, especially for accommodating IC local circuit neurons, was a primary outcome of such neuromodulation therapy. Future studies, using direct *in vivo* assessment of subpopulations of IC activity, should consider which of the IC neural populations (afferent, efferent or local circuit neurons; (Beaumont *et al.*, 2013)) are impacted by chronic SCS, particularly with regard to their intra-ganglionic interactions and the potential for phenotype reorganization of impacted IC neurons (Armour, 2008; Beaumont *et al.*, 2013; Hardwick *et al.*, 2008; Hoover *et al.*, 2009; Parsons, 2004).

Study limitations

It is hypothesized that IC network synaptic efficacy underpins AF protection, but we directly evaluated the effects of chronic SCS on this only at the 5 week time point. While the efficacy for SCS mediated anti-anginal therapy demonstrates persistence (Mannheimer *et al.*, 2002), it remains to be determined as to the temporal changes in IC network processing with respect to control of the mechanical and electrical substrate in both the normal and diseased states. It should also be considered that SCS has the potential to impact cardiomyocytes directly thereby rendering them stress resistant (Southerland *et al.*, 2007) and, as such, contributing to the overall electrical stability of the stressed heart.

Perspectives

Data derived from this study indicate that the antiarrhythmic benefits that long-term SCS impart against neurogenic atrial tachyarrhythmias cannot be attributed to direct modification of the electrophysiological properties of somata within the ICNS. Rather, this form of therapy appears to target the capacity of the ICNS to transduce excessive inputs - in this instance from extracardiac neuronal sources – to regularize its target organ control. Thus, while individual neurons with the ICNS may not be directly modified by long term SCS therapy, their collective integrative capacity to transduce excessive inputs appears to represents the main target of such chronic therapy in the suppression of neurogenically derived atrial tachyarrhythmias. Lopshire et al demonstrated an anti-arrhythmic effect to chronic SCS in a post-infarct heart failure model, a response likely related to modulation of the IC networks (Lopshire *et al.*, 2009). That the properties of neuronal somata *per se* do not appear to be compromised by such neuromodulation therapy suggests its therapeutic safety given the physiological importance of intrinsic cardiac neurons in cardiac function.

Acknowledgments

The authors wish to thank Ms. Caroline Bouchard for her expert technical assistance in the performance of the experiments.

Funding: This work was supported by grants from the Canadian Institutes of Health Research (MOP-11252 to R.C., F.M.S), the National Institutes of Health (RO1 HL71830 to JLA), and St. Jude Medical/Advanced Neuromodulation Systems.

Reference List

Ajijola OA, Yagishita D, Patel KJ, Vaseghi M, Zhou W, Yamakawa K, So E, Lux RL, Mahajan A, Shivkumar K. Focal myocardial infarction induces global remodeling of cardiac sympathetic innervation: neural remodeling in a spatial context. Am J Physiol Heart Circ Physiol. 2013; 305:H1031–H1040. [PubMed: 23893167]

Ardell, JL. Intrathoracic neuronal regulation of cardiac function. In: Armour, JA.; Ardell, JL., editors. Basic and Clinical Neurocardiology. Oxford University Press; New York: 2004. p. 118-152.

Armour JA. Potential clinical relevance of the ‘little brain’ on the mammalian heart. Exp Physiol. 2008; 93(2):165–176. [PubMed: 17981929]

Armour, JA.; Kember, G. Cardiac Sensory Neurons. In: Armour, JA.; Ardell, JL., editors. Basic and Clinical Neurocardiology. Oxford Univ. Press; NY: 2004. p. 79-117.

Armour JA, Linderoth B, Arora RC, DeJongste MJL, Ardell JL, Kingma JG, Hill M, Foreman RD. Long-term modulation of the intrinsic cardiac nervous system by spinal cord neurons in normal and ischemic hearts. Autonomic Neuroscience: Basic and Clinical. 2002; 95:71–79. [PubMed: 11873770]

Armour JA, Richer LP, Pagé PL, Vinet A, Kus T, Vermeulen M, Nadeau R, Cardinal R. Origin and pharmacological response of atrial tachyarrhythmias induced by activation of mediastinal nerves in canines. Autonomic Neuroscience: Basic and Clinical. 2005; 118:68–78. [PubMed: 15795179]

Beaumont E, Salavatian S, Southerland EM, Vinet A, Jacquemet V, Armour JA, Ardell JL. Network interactions within the canine intrinsic cardiac nervous system: Implications for reflex control of regional cardiac function. J Physiol. 2013; 591:4515–4533. [PubMed: 23818689]

Bernstein SA, Wong B, Vasquez C, Rosenberg SP, Rooke R, Kuznekoff LM, Lader JM, Mahoney VM, Budylin T, Alvstrand M, Rakowski-Anderson T, Bharmi R, Shah R, Fowler S, Holmes D, Farazi TG, Chinitz LA, Morley GE. Spinal cord stimulation protects against atrial fibrillation induced by tachypacing. Heart Rhythm. 2012; 9:1426–1433. [PubMed: 22554859]

Brunton, LL.; Chabner, BA.; Knollmann, BC. Goodman & Gillmans Pharmacological Basis of Therapeutics. 12. 2010.

Cardinal R, Ardell JL, Linderoth B, Vermeulen M, Foreman RD, Armour JA. Spinal cord activation differentially modulates ischemic electrical responses to different stressors in canine ventricles. Autonomic Neuroscience: Basic and Clinical. 2004; 111:37–47. [PubMed: 15109937]

Cardinal R, Armour JA, Bouchard C, Vermeulen M, Vinet A, Nadeau R, Page P. Atrial tachyarrhythmias and repolarization changes induced by discrete activation of dorsal mediastinal cardiac nerves in canines. Circ Arrhythm Electrophysiol. 2010; 3:511–520. [PubMed: 20693578]

Cardinal R, Pagé PL, Vermeulen M, Bouchard C, Ardell JL, Foreman RD, Armour JA. Spinal cord stimulation suppresses bradycardias and atrial tachyarrhythmias induced by mediastinal nerve stimulation in dogs. Am J Physiol Regulatory Integrative Comp Physiol. 2006; 291:R1369–R1375.

Chen P-S, Tan AY. Autonomic nerve activity and atrial fibrillation. Heart Rhythm. 2007; 4:S61–S64. [PubMed: 17336887]

Dell'Italia LJ. Translational success stories: angiotensin receptor 1 antagonists in heart failure. Circ Res. 2011; 109:437–452. [PubMed: 21817164]

Eliasson T, Augustinsson LE, Mannheimer C. Spinal cord stimulation in severe angina pectoris: Presentation of current studies, indications and clinical experience. Pain. 1996; 65:169–179. [PubMed: 8826504]

Foreman RD, Linderoth B, Ardell JL, Barron KW, Chandler MJ, Hull SS, TerHorst GJ, DeJongste MJL, Armour JA. Modulation of intrinsic cardiac neurons by spinal cord stimulation: implications for therapeutic use in angina pectoris. Cardiov Res. 2000; 47:367–375.

Gibbons DD, Southerland EM, Hoover DB, Beaumont E, Armour JA, Ardell JL. Neuromodulation targets intrinsic cardiac neurons to attenuate neuronally mediated atrial arrhythmias. Am J Physiol Regul Integr Comp Physiol. 2012; 302:R357–R364. [PubMed: 22088304]

Hardwick JC, Southerland EM, Ardell JL. Chronic myocardial infarction induces phenotypic and functional remodeling in the guinea pig cardiac plexus. Am J Physiol Regulatory Integrative Comp Physiol. 2008; 295:R1926–R1933.

Hoover DB, Isaacs ER, Jacques F, Hoard JL, Pagé P, Armour JA. Localization of multiple neurotransmitters in surgically derived specimens of human atrial ganglia. Neurosci. 2009; 164:1170–1179.

Kember G, Armour JA, Zamir M. Neural Control Hierarchy Of The Heart Has Not Evolved To Deal With Myocardial Ischemia. Physiol Genomics. 2013; 45:638–644. [PubMed: 23695889]

Liu Y, Yue WS, Liao SY, Zhang Y, Au KW, Shuto C, Hata C, Park E, Chen P, Siu CW, Tse HF. Thoracic spinal cord stimulation improves cardiac contractile function and myocardial oxygen consumption in a porcine model of ischemic heart failure. J Cardiovasc Electrophysiol. 2012; 23:534–540. [PubMed: 22151312]

Longhurst JC, Tjen-A-Looi SC, Fu L-W. Cardiac sympathetic afferent activation provoked by myocardial ischemia and reperfusion. New York Acad of Sciences. 2001; 940:74–95.

Lopshire JC, Zhou X, Dusa C, Ueyama T, Rosenberger J, Courtney N, Ujhelyi M, Mullen T, Das M, Zipes DP. Spinal cord stimulation improves ventricular function and reduces ventricular arrhythmias in a canine postinfarction heart failure model. Circulation. 2009; 120:286–294. [PubMed: 19597055]

Lopshire JC, Zipes DP. Device therapy to modulate the autonomic nervous system to treat heart failure. Curr Cardiol Rep. 2012; 14:593–600. [PubMed: 22833301]

Mannheimer C, Camici P, Chester MR, Collins A, DeJongste MJL, Eliasson T, Follath F, Hellemans I, Herlitz J, Lüscher T, Pasic M, Thelle D. The problem of chronic refractory angina. Report from the ESC joint study group on the treatment of refractory angina. Eur Heart J. 2002; 23:355–370. [PubMed: 11846493]

Nakahara S, Tung R, Ramirez RJ, Michowitz Y, Vaseghi M, Buch E, Gima J, Wiener I, Mahajan A, Boyle NG, Shivkumar K. Characterization of the arrhythmogenic substrate in ischemic and nonischemic cardiomyopathy implications for catheter ablation of hemodynamically unstable ventricular tachycardia. J Am Coll Cardiol. 2010; 55:2355–2365. [PubMed: 20488307]

Parsons, RL. Mammalian cardiac ganglia as local integration centers: histochemical and electrophysiological evidence. In: Dun, NJ.; Machado, BH.; Pilowsky, PM., editors. Neural

Mechanisms of Cardiovascular Regulation. Kluwer Academic Publishers; Boston: 2004. p. 335-356.

Richer LP, Vinet A, Kus T, Cardinal R, Ardell JL, Armour JA. α–Adrenoceptor blockade modifies neurally induced atrial arrhythmias. Am J Physiol Regulatory Integrative Comp Physiol. 2008; 295:R1175–R1180.

Schwartz PJ. Vagal stimulation for the treatment of heart failure: a translational success story. Heart. 2012; 98:1687–1689. [PubMed: 22962285]

Southerland EM, Milhorn D, Foreman RD, Linderoth B, DeJongste MJL, Armour JA, Subramanian V, Singh M, Singh K, Ardell JL. Preemptive, but not reactive, spinal cord stimulation mitigates transient ischemia-induced infarction via cardiac adrenergic neurons. Am J Physiol Heart Circ Physiol. 2007; 292:H311–H317. [PubMed: 16920800]

Tjen-A-Looi SC, Bonham A, Longhurst JC. Interactions between sympathetic and vagal cardiac afferents in nucleus tractus solitarii. Am J Physiol Heart Circ Physiol. 1997; 272:H2843–H2851.

Zucker IH, Patel KP, Schultz HD. Neurohumoral stimulation. Heart Fail Clinics. 2012; 8:87–99.

Highlights

- Spinal cord stimulation (SCS) suppresses neurally induced atrial fibrillation (AF)
- Effectiveness of SCS in AF suppression increases with time
- SCS minimally impacts active and passive properties of individual intrinsic cardiac neurons
- SCS modifies synaptic efficacy of the IC network
- SCS differentially impacts the neurotransmission to the accommodating sub-population of IC neurons

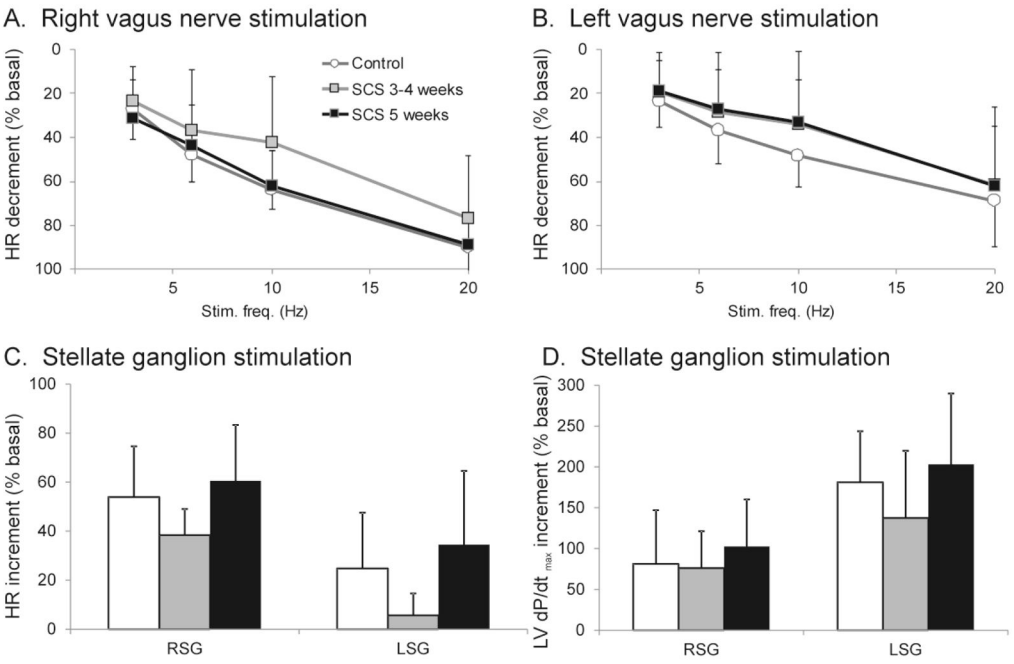

Figure 1.

Chronic SCS does not functionally remodel chronotropic or inotropic responses to sympathetic or parasympathetic efferent neuronal stimulation. Percent change in heart rate (panels A–C) or left ventricular dp/dt $_{max}$ (panel D) in response to right (panel A) or left (panel B) cervical vagosympathetic vs right (RSG) or left (LSG) stellate ganglia (panels C–D) stimulation in sham controls versus animals with 3–4 versus 5 weeks of chronic SCS. Data are expressed as mean ± SD.

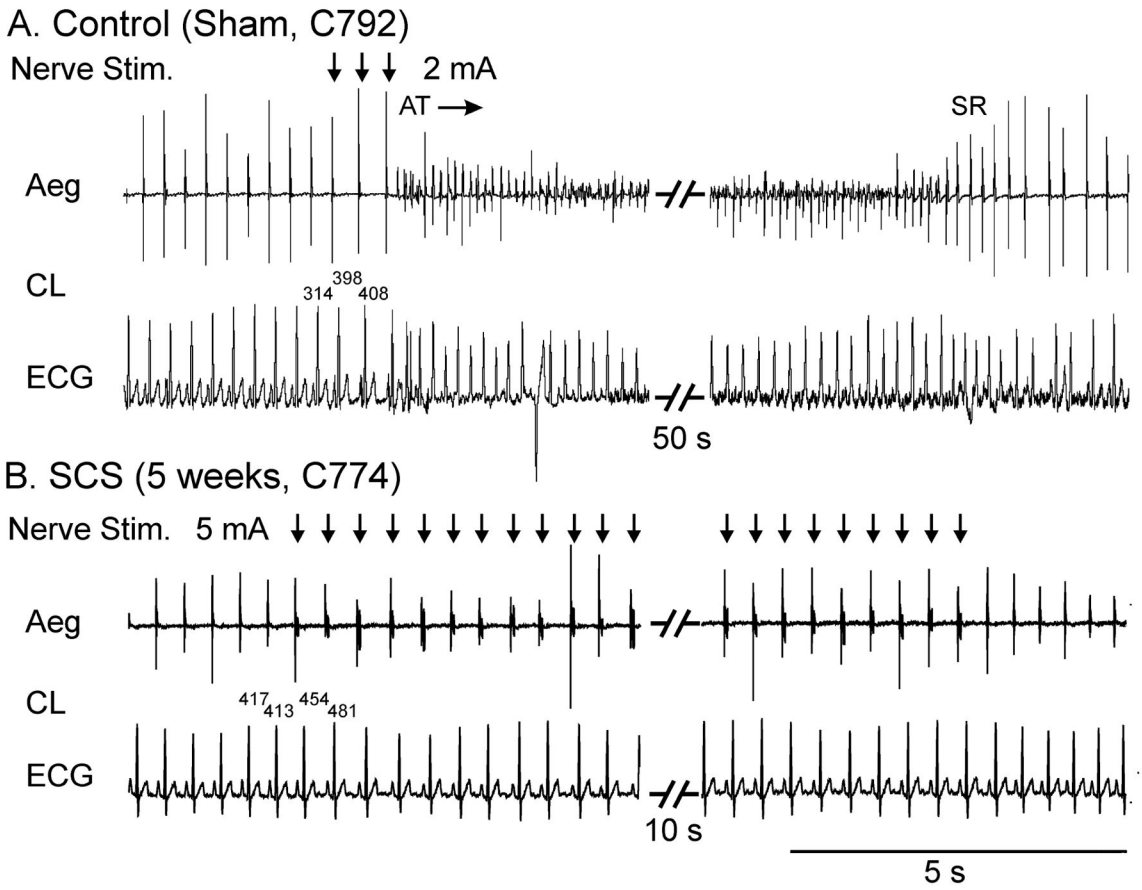

Figure 2.

Chronic SCS suppresses atrial tachyarrhythmias of neural origin. **A**. In a control (sham SCS) preparation, delivering 3 stimuli (current intensity: 2 mA; vertical arrows) to a mediastinal nerve first prolonged atrial cycle length (CL) that rapidly transitioned to an atrial arrhythmia episode that lasted for 58 s before converting spontaneously to sinus rhythm (SR). **B.** In an animal exposed to 5 weeks of SCS, sinus CL prolongation was elicited during mediastinal nerve stimulation - with no accompanying AT episode. Abbreviations: Aeg = atrial electrogram; cycle length = atrial electrical cycle duration; ECG = electrocardiogram; mA = current.

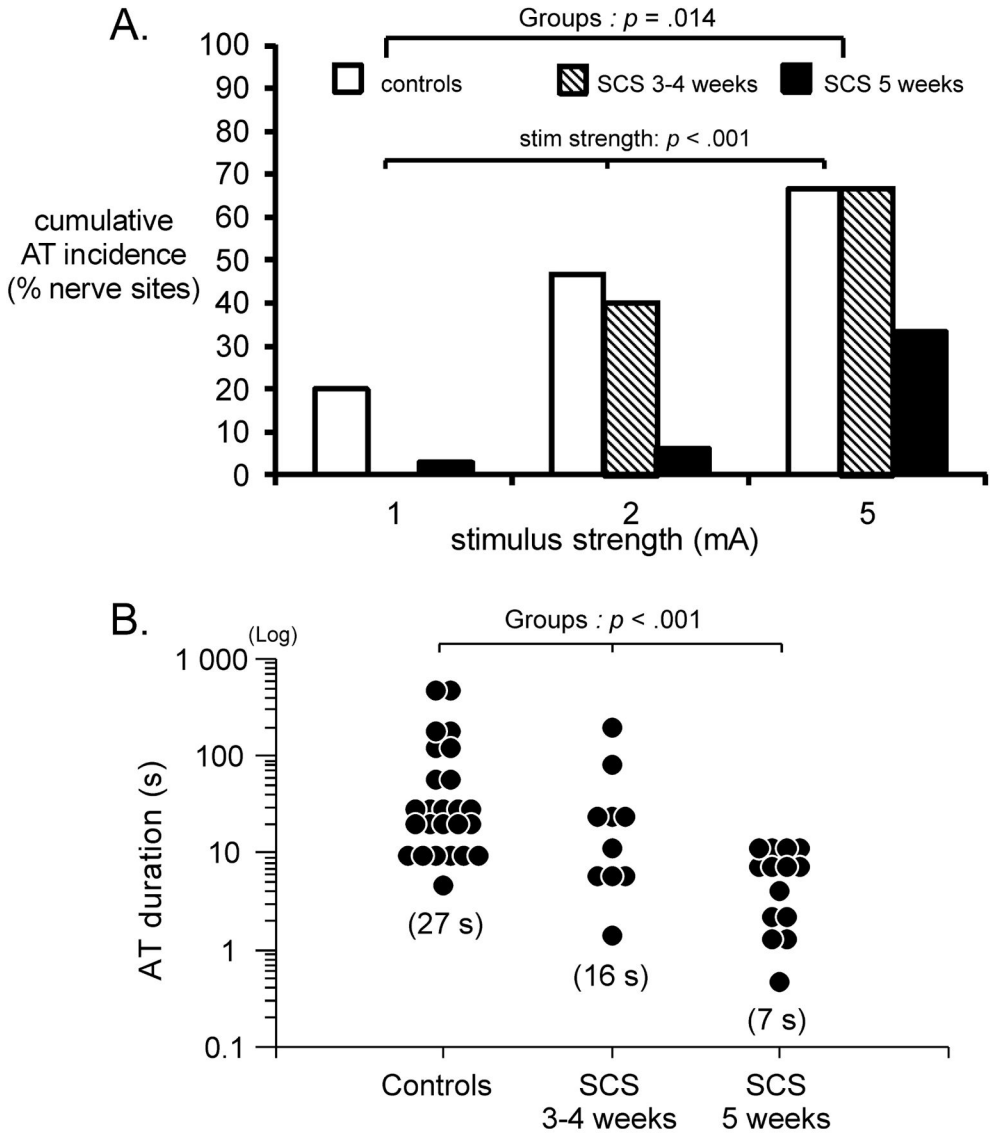

Figure 3.

A. Cumulative indices of atrial tachyarrhythmias (AT) induced in the three groups, as engendered with increasing mediastinal nerve stimulus strengths (1, 2, and 5 mA). Controls: AT incidence increased from 20% at 1 mA (6/30) to 46% at 2 mA (14/30) and to 66% at 5 mA (20/30). Incidences decreased following 3–4 weeks (cross-hatched bar: 5 preparations) and even more following 5 weeks (filled bar: 11 preparations) of SCS. **B**. The durations of ATs induced among controls (25 episodes) (median values of 27s) were reduced to 16s (SCS 3–4 weeks) and 7s (SCS 5 weeks) ($p<.001$, group differences demonstrated by Kruskal-Wallis one-way analysis of variance for independent samples) by SCS.

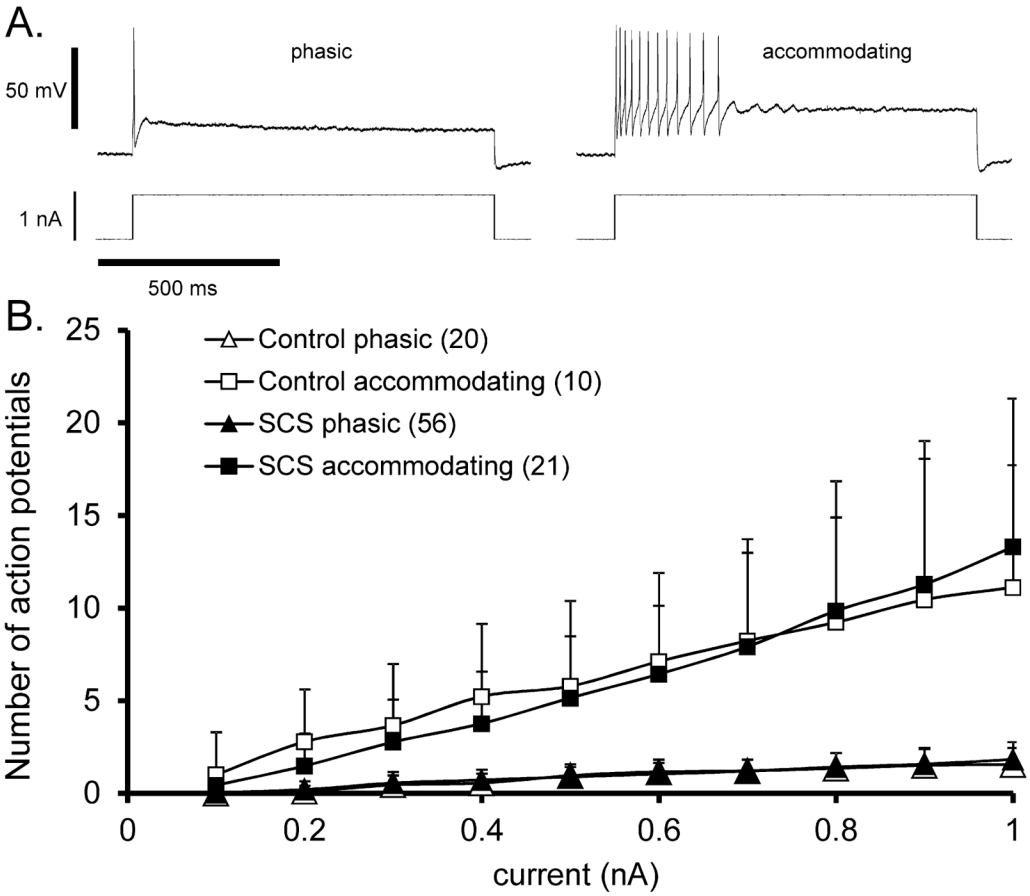

Figure 4.

IC soma excitability, as assessed by transient depolarizing currents, and stratification into phasic versus accommodating neuron types dependent upon evoked responses (A) was unaltered by chronic SCS (B). Numbers in parentheses indicate number of neurons. nA=nanoampere.

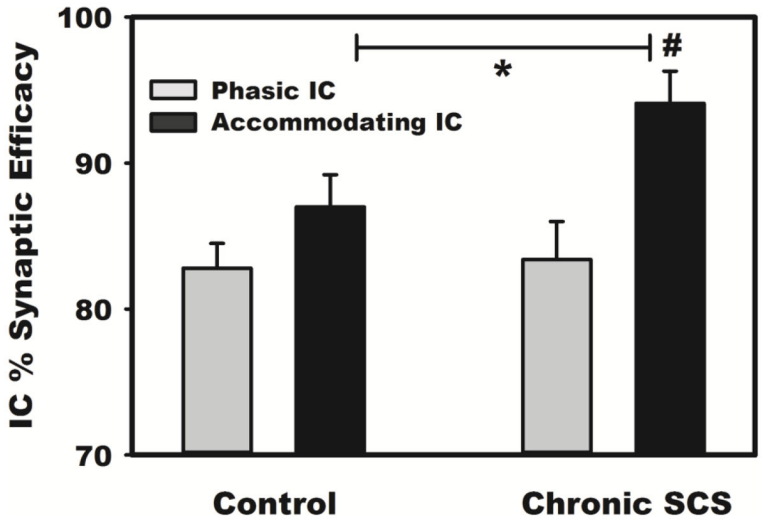

Figure 5.

IC neuronal response (i.e. soma action potential generation) to stimulation of adjacent intra-ganglionic axonal inputs. Control and chronic SCS derived IC somata responses to 2–20 Hz stimuli (0.5 ms duration; current 2x threshold) were grouped based on classification as phasic or accommodating as described in Figure 4. Synaptic efficacy, as defined by % action potential output/stimulus pulse input frequency, was differentially enhanced in accommodating IC neurons in chronic SCS. * $p<0.04$ between treatment group (control vrs SCS); # $p<0.008$ phasic vs accommodating IC neurons.

Table 1

Membrane properties and regenerative responses to intracellular current injection.

	n	I_t nA	R_{in} MΩ	RMP mV	ΔV_t mV	AP ampl mV	AP dur ms	AHP ampl mV	AHP dur ms
Control phasic	20	0.43 ± 0.17	45 ± 45	−50 ± 5	21 ± 5	61 ± 14	1.5 ± 0.5	12 ± 3	19 ± 13
Control accom	11	*0.16 ± 0.05	*103 ± 76	−46 ± 6	20 ± 6	63 ± 17	1.6 ± 0.4	15 ± 5	31 ± 21
SCS phasic	55	0.40 ± 0.19	25 ± 24	−47 ± 7	20 ± 4	57 ± 10	1.5 ± 0.3	13 ± 3	20 ± 11
SCS accom	21	*0.20 ± 0.07	*82 ± 60	−52 ± 9	20 ± 3	54 ± 11	1.5 ± 0.4	14 ± 4	27 ± 19

Values are expressed as mean ± 1 standard deviation. Abbreviations. accom: accommodating neuron; I_t: threshold current; R_{in}: whole-cell input resistance; RMP: resting membrane potential; AP ampl: action potential amplitude; AP dur: action potential duration; AHP ampl: after hyperpolarization amplitude; AHP dur: after hyperpolarization duration; nA: nanoampere; MΩ: megohm; mV: millivolt; ms: millisecond.

*$p \leq .05$ comparing neuron types within each group.