Increased intrinsic excitability of muscle vasoconstrictor preganglionic neurons may contribute to the elevated sympathetic activity in hypertensive rats

Running heading: Reduced I_A in sympathetic preganglionic neurons of SH rat

Author list: Linford JB Briant1,3, Alexey O Stalbovskiy1, Matthew F Nolan4, Alan R Champneys3, Anthony E Pickering1,2

1 - School of Physiology & Pharmacology, Medical Sciences Building, University Walk, University of Bristol, Bristol, BS8 1TD, UK. 2 - Department of Anaesthesia, University Hospitals Bristol, Bristol, BS2 8HW, UK. 3 - Department of Engineering Mathematics, Merchant Venturers Building, Woodland Road, University of Bristol, Bristol, BS8 1UB, UK. 4 - Centre for Integrative Physiology, University of Edinburgh, Hugh Robson Building, George Square, Edinburgh, EH8 9XD

Author contributions:
All authors contributed to the conception and design of the research
All authors interpreted the results of the experiments
All authors edited and revised the manuscript
All authors have approved the final version of the manuscript
LJBB, AOS and AEP performed the experiments
LJBB, AOS, ARC and AEP analyzed the data
LJBB, AOS and AEP prepared the figures
LJBB, ARC and AEP drafted the manuscript

Word Count: 12,660

Author for correspondence: A.E. Pickering, School of Physiology & Pharmacology, School of Medical Sciences, University Walk, University of Bristol, Bristol, BS8 1TD, UK. e-mail: Tony.Pickering@Bristol.ac.uk; Tel: +44 (0)117 331 2311; Fax: +44 (0)117 331 2288

Copyright © 2014 by the American Physiological Society.
Abstract

Hypertension is associated with pathologically increased sympathetic drive to the vasculature. This has been attributed to increased excitatory drive to sympathetic preganglionic neurons (SPN) from brainstem cardiovascular control centers. However, there is also evidence supporting increased intrinsic excitability of SPN. To test this hypothesis, we made whole-cell recordings of muscle vasoconstrictor (MVC\textsubscript{like}) SPN in the working-heart brainstem preparation of spontaneously hypertensive (SH) and normotensive Wistar-Kyoto (WKY) rats. The MVC\textsubscript{like} SPN have a higher spontaneous firing frequency in the SH rat (3.85±0.4 vs 2.44±0.4Hz in WKY; p=0.011) with greater respiratory modulation of their activity. The action potentials of SH SPN had smaller, shorter AHPs and showed diminished transient rectification indicating suppression of an A-type potassium conductance ($I_A$). We developed mathematical models of the SPN to establish if changes in their intrinsic properties in SH rats could account for their altered firing. Reduction of the maximal conductance density of $I_A$ by 15-30%, changed the excitability and output of the model from the WKY to a SH profile, with increased firing frequency, amplified respiratory modulation and smaller AHPs. This change in output is predominantly a consequence of altered synaptic integration. Consistent with these \textit{in silico} predictions, we found that intrathecal 4-AP increased sympathetic nerve activity, elevated perfusion pressure and augmented Traube-Hering waves. Our findings indicate that $I_A$ acts as a powerful filter on incoming synaptic drive to SPN and that its diminution in the SH rat is potentially sufficient to account for the increased sympathetic output underlying hypertension.

\textbf{Key words:} sympathetic preganglionic, vasomotor tone, hypertension, transient rectification.
Introduction

Sympathetic activity is elevated in hypertensive patients, in pre-hypertensive conditions and in animal models of hypertension (reviewed in (Esler 2011; Fisher and Paton 2012; Grassi 1998)). This has recently led to trials of novel therapeutic interventions aimed at reducing the sympathetic over-activity, for example renal nerve denervation (Schlaich et al. 2009) and carotid sinus stimulation (Jordan et al. 2012). Notwithstanding these advances, hypertension remains a common clinical problem and despite a range of drug treatments a substantial population of hypertensive patients (~14%) remain refractory to therapy and at risk of cardiovascular morbidity (Carey 2013). Therefore there is an imperative to better understand the factors leading to the increased sympathetic outflow.

The sympathetic outflow is specialized according to the target organs and the muscle vasoconstrictor class (MVC) of sympathetic neuron is believed to be particularly important in the control of blood pressure (Janig 2006). These sympathetic vasoconstrictor pathways produce a tonic release of noradrenaline that maintains vascular tone. This tonic sympathetic activity originates in supraspinal structures including the rostro-ventrolateral medulla (RVLM) and the hypothalamic paraventricular nucleus (reviewed in (Guyenet 2006)). Mechanisms suggested to account for the elevation of sympathetic nerve activity seen in hypertension have focused on these brainstem and higher centers (Moraes et al. 2014; Sved et al. 2003) or on cardiorespiratory afferent inputs (DiBona and Esler 2010; McBryde et al. 2013). Intriguingly there have been reports of increased sympathetic excitability at a spinal level in spontaneously hypertensive rats that is maintained after removal of inputs to the spinal cord (Schramm and Barton 1979; Schramm and Chornoboy 1982; Schramm et al. 1979). As yet the cellular mechanisms for this spinally mediated increase in sympathetic discharge have received relatively little attention.
The intrinsic membrane properties of SPN may be important determinants of the sympathetic activity received by blood vessels as they have powerful rectifying conductances - including a prominent transient rectification (Dembowsky et al. 1986; Miyazaki et al. 1996; Pickering et al. 1991; Whymant et al. 2011; Yoshimura et al. 1987) - that contribute to their relatively low (2-3Hz) firing frequency, in response to the high frequency (>20Hz) of ongoing synaptic inputs (Stalbovskiy et al. 2014). We therefore set out to investigate the possibility that altered excitability of SPN accounts for increased sympathetic activity in hypertension. To test this hypothesis we obtained whole-cell recordings of MVC-like SPN in the working heart brainstem preparation (Paton 1996) – wherein the SPN can be functionally characterized by their responses to cardiorespiratory reflex activation (Stalbovskiy et al. 2014). This allowed us to characterize both the intrinsic properties and network drives of SPN in spontaneously hypertensive (SH) rats and also normotensive WKY rats. We undertook these studies in neonatal animals (p7-16) - before they have developed hypertension - allowing us to detect changes in excitability that could be causal rather than simply associative.

We find that the firing frequency of MVC-like SPN is increased in the SH rat with an exaggerated respiratory-sympathetic modulation, findings that echo the whole sympathetic nerve recordings of Simms et al. (2010; 2009). This is associated with a diminution in their transient rectification, but no apparent change in the incoming synaptic input to SPN. We therefore built a conductance-based model of a MVC-like SPN in the NEURON environment (Hines et al. 2004) with particular focus on achieving biophysically accurate kinetics of IA (Bordey et al. 1995; Whyment et al. 2011). We show that varying the conductance density of IA replicates the increased sympathetic output and altered excitability seen in our recordings without a requirement for a change in the afferent drive. Further we show that intrathecal administration of 4-aminopyridine to block the A-current at a spinal level in situ (Pickering
and Paton 2006; Sadananda et al. 2011), produces a dramatic increase in the level of sympathetic activity consistent with it playing a substantial role in gating the sympathetic outflow.

**Glossary**

\( \bar{g}_A \) Maximal conductance density of \( I_A \) (mS/cm²)

\( k_n \) Activation slope factor of \( I_A \) (mV⁻¹)

\( k_l \) Inactivation slope factor of \( I_A \) (mV⁻¹)

\( V_M \) Membrane potential (mV)

\( V_{RI} \) Repolarization inflection potential (mV)

\( V_{\bar{z}_n} \) Half-activation of \( I_A \) (mV)

\( V_{\bar{z}_l} \) Half-inactivation of \( I_A \) (mV)

\( \zeta_n \) Valence of activation gate of \( I_A \) (mV⁻¹)

\( \zeta_l \) Valence of inactivation gate of \( I_A \) (mV⁻¹)
Experimental Methods

All experiments conformed to the UK Home Office guidelines regarding the ethical use of animals and were approved by the University of Bristol ethical review committee. Male Wistar-Kyoto rats (WKY; n=34, p7-16) and spontaneously hypertensive (SH (Okamoto and Aoki 1963); n=32, p8-16) rats were used in the cell recording studies and WKY rats (n=6, p21-24) were used for the sympathetic nerve recordings.

Working Heart Brainstem Preparation

The working heart brainstem preparation (WHBP) was used for all patch clamp recordings of SPN in the lateral horn of the spinal cord (Stalbovskiy et al. 2014). In brief, rats were deeply anesthetized with halothane, until loss of withdrawal to paw pinch. The rat was bisected sub-diaphragmatically, exsanguinated, cooled in Ringer’s solution at 5ºC, and suction decerebrated pre-collicularly following which the halothane anesthesia was discontinued. The preparation was kept cold while the phrenic nerve and descending aorta were dissected free and a bilateral pneumonectomy was performed. The preparation was positioned prone while still cold and access to the spinal cord was obtained via a laminectomy up to the level of C7. The dura was incised and the dorsal pia mater was removed locally at the level of T3. A single cut was made in the spinal cord using a custom built piezoslicer (Smith et al. 2007). This employed a piezoelectric bending actuator (Piezo Systems, Woburn, MA, USA) with a microblade (FST 10035-05) to produce the “slice in situ” preparation with a 45º bevel on the cut end of the cord at the level of T3 for recordings. The preparation was transferred to a recording chamber in ear bars and positioned prone to allow access to the cut surface of the spinal cord slice. A double lumen cannula (Ø 1.25 mm, DLR-4, Braintree Scientific, MA, USA) was inserted into the descending aorta for retrograde
perfusion with carbogen-gassed, modified Ringer’s solution (see below for composition) containing Ficoll-70 (1.25%; Sigma) at 30°C. The perfusion pressure was monitored via the second lumen of the cannula. The heart resumed beating almost immediately as the perfusate flow was commenced (11-13 ml/min) and rhythmic respiratory muscle contractions commenced after 1-3 min, signaling the return of brainstem function. At this point muscle relaxant was added to the perfusion solution (Vecuronium 200 mcg; Norcuron, Organon, Cambridge, UK) to allow stable recordings.

Phrenic nerve activity was recorded using a glass suction electrode to give a physiological index of preparation viability. The signal from the phrenic nerve was AC amplified and band pass filtered (80Hz-3KHz). The perfusion pressure was adjusted to obtain an optimal eupnoeic pattern of PNA by addition of vasopressin (2-400pM, Sigma) to the reservoir and/or increase of the pump flow rate. Chlorided silver electrodes were inserted bilaterally into the rib cage to record ECG allowing instantaneous heart rate to be derived.

**Decerebrate arterially-perfused rat preparation**

The decerebrate arterially-perfused rat (DAPR) preparation was used to examine the effect of intrathecal 4-aminopyridine upon the sympathetic outflow and was set up using previously described methods (Pickering and Paton 2006; Sadananda et al. 2011). In brief, WKY rats (40-90g, P21-24) were heparinized (100 IU, i.p.) before being deeply anesthetized with halothane, until loss of withdrawal to paw pinch. Following a midline laparotomy, the stomach, spleen and free intestine were vascularly isolated with ligatures and removed. The animal was immediately cooled by immersion in Ringer’s (5°C, composition below) and decerebrated, by aspiration, at the pre-collicular level to render it insentient (at this point the halothane was withdrawn).

After skin removal and a midline sternotomy the thoracic cavity was opened with insertion of a spreading retractor. The left phrenic nerve was identified and the lungs and diaphragm
were removed. Both atria were incised to avoid venous congestion during subsequent arterial
perfusion. An incision was made at the apex of the heart for insertion of the perfusion
cannula into the ascending aorta. A single segment laminectomy allowed an intrathecal 32-
gauge intrathecal catheter (CR3212; ReCathCo; Allison Park; PA) to be threaded through a
25-gauge hypodermic needle under direct vision to sit at a low thoracic level.

The preparation was transferred to the recording chamber and a double lumen cannula
was inserted into the ascending aorta. The preparation was arterially perfused (flow rate ~
30ml/min), optimized and the phrenic nerve recorded as for the WHBP (above). Recordings
from the thoracic sympathetic chain were obtained using a bipolar suction electrode at the
level of T12 and were AC amplified and band pass filtered (100Hz-3KHz).

Whole-Cell recordings from Sympathetic Preganglionic Neurons

The outline of the lateral horn was clearly visible under a binocular microscope (Leica MZ-
6) on the cut face of the cord, allowing the recording patch electrode to be directed into the
SPN cell column. Blind, whole cell recordings were made from neurons at depths of 50-
500µm below the cut surface. Electrodes were pulled from borosilicate capillaries (GC150-
TF10, Harvard Apparatus, MA, USA) to have a resistance of 5-10MΩ. Stable gigaohm seals
and subsequent whole cell recordings were obtained from neurons for periods of over 1 hour
with access resistances of 20-50MΩ.

Current clamp and voltage clamp recordings were made using a discontinuous clamp
amplifier (SEC-05LX-BF, NPI electronic, Tamm, Germany) with switching frequencies
between 10 and 15KHz and a 25% duty cycle after optimization of capacitance
compensation. The gain was maximally increased to just below the point of clamp instability
(typically ~1000x) as assessed from continuous monitoring of the electrode potential output.
Cell recordings were low pass filtered at 2Kz and the signal passed through a Humbug
(Digitimer, UK) to remove mains interference. Data was sampled at 5 KHz using a power1401 A-D converter (CED, UK).

Lateral horn neurons were definitively identified as being SPN by antidromic activation following stimulation (0.3-1ms, 5-20V, 0.2-20Hz) of the ventral root exit zone of the spinal cord with a concentric bipolar electrode (SNE100, Rhodes Medical Instruments, CA, USA). Cancellations were sought by depolarizing the cell to fire spontaneous action potentials while applying ventral root stimuli. A total of 43/64 (67.2%) of the SPN tested were definitively identified antidromically. The remaining cells were identified as SPN on the basis of characteristic electrophysiology, post-hoc anatomical reconstruction and by their responses to functional cardiorespiratory reflex activation (Stalbovskiy et al. 2014).

**Cardiorespiratory reflexes**

In each neonatal WHBP preparation the afferent stimulus was titrated at the start of the experiment to reproducibly evoke the expected physiological responses. Peripheral chemoreceptors were stimulated using intra-arterial injection of sodium cyanide (50-100ul of 0·03%) as a bolus into the perfusion line. The chemoreflex responses were dose-dependent and the doses used produced sub-maximal bradycardia (1-2Hz) and hyperpnoea. The diving response was evoked by application of cold Ringer’s (∼10°C, 50-200ul) to the snout that triggered a characteristic apnoea (lasting for > 2x basal respiratory cycle period) and transient bradycardia.

**SPN recording protocol**

Following seal rupture the initial recordings were made in current clamp mode and the cell was allowed to stabilize before:

i. baseline current clamp recording of firing activity (~1min)

ii. diving response and peripheral chemoreflex activation.
iii. voltage responses to injection of current pulses

iv. antidromic stimulation

v. voltage clamp to resolve synaptic events underlying the basal firing

Experimental data Recording & Analysis

Perfusion pressure, electrocardiogram and phrenic nerve activity were recorded using custom built AC amplifiers and transducers (designed and built by Jeff Croker, University of Bristol) and collected via an A-D interface (power1401, CED, Cambridge, UK) to a computer running Spike2 software (CED, Cambridge, UK). Custom scripts were used for data acquisition and analysis in Spike2.

All membrane potentials were corrected for a junction potential of 13mV. SPN with resting membrane potentials greater than -40mV and whose action potentials overshot zero were included for the analysis of membrane properties. Spike parameters were measured from spontaneous action potentials and the threshold for spike discharge was taken arbitrarily as the point at which the rate of rise of membrane potential exceeded 7.5V.s\(^{-1}\). The spike parameters were measured with reference to this threshold point. Spike amplitude was measured above threshold and duration was calculated at 1/3 of spike amplitude. The duration of the AHP was calculated from where spike repolarization crossed threshold to the point of return to resting potential. The AHP amplitude was measured from spike threshold to the trough. The input resistance and time constant were estimated from the voltage deflection (amplitude 5-10mV) in response to small hyperpolarizing current pulses (5-20pA applied for 1s).
Statistical Analysis

Data are expressed as mean ± standard error or median [interquartile range]. n refers to the number of cells. Two tailed t-tests or ANOVA were used to establish statistical significance (Prism 5, GraphPad Software, San Diego, USA) defined as P<0·05.

Drugs and solutions

The composition of the modified Ringer’s solution used as perfusate was (mM): NaCl (125); NaHCO₃ (24); KCl (3); CaCl₂ (2·5); MgSO₄ (1·25); KH₂PO₄ (1·25); dextrose (10); pH 7·35-7.4 after carbogenation. The patch solution contained (mM) KGlucose - 130; KCl - 10; NaCl - 10; MgCl₂ - 2; HEPES - 10; NaATP - 2; NaGTP - 0.2 (pH 7.4 and osmolarity - 300mOsm). All chemicals were from Sigma (UK).

Computational Overview

A quantitative model of a MVC-like SPN was constructed within the simulation environment NEURON v7.3 (Carnevale 2006); code for model now deposited on ModelDB (senselab.med.yale.edu/modeldb; accession number: 151482).

Model Cell Morphology

The model SPN (see Figure 4) was based on experimental data (Forehand 1990; Sah and McLachlan 1995) and had an ovoid soma with dimensions 25×15μm with 3 lateral dendrites (200μm long x 2μm diameter, 10 segments) and a single medial primary dendrite (25μm x 5μm, 5 segments) from which two secondary medial dendrites emerge (600μm x 2μm, 20 segments). A single unbranched axon arises from the soma (length 500μm and diameter of 0.5μm, 20 segments). The axial resistance was 120Ω.cm and the membrane capacitance was 1μF.cm⁻².
Membrane Properties

The passive electrophysiological properties of SPNs in the rat have been reported from numerous *in vitro* studies, with resting membrane potentials of approximately -55mV (Logan et al. 1996; Miyazaki et al. 1996; Pickering et al. 1991; Sah and McLachlan 1995; Whyment et al. 2011; Wilson et al. 2002; Yoshimura et al. 1986a; b). The reversal potential and maximal conductance density of the leak conductance were set to $E_{\text{pas}}=-40\text{mV}$ and $g_{\text{pas}}=0.018\text{mS.cm}^{-2}$ in the soma, to adjust both the resting membrane potential and input resistance to be within the physiological range. The input resistance ($R_m$) was measured as 320.9M$\Omega$ in keeping with experimental data (Sah and McLachlan 1995; Stalbovskiy et al. 2014; Wilson et al. 2002).

The model included passive, voltage-dependent and calcium-dependent conductances (Figure 4) selected based on experimental evidence for their involvement in determining membrane excitability close to the resting potential (see Appendix table 1). The parameters for the voltage-gated channels (see appendix for full descriptions) were based on previously published experimental and modeling studies (Migliore et al. 1995; Migliore et al. 1999). This study focused on the influence of the A-current on SPN excitability across the rat strain and as such the parameters for this conductance were fitted to the existing experimental data (Dembowsky et al. 1986; Miyazaki et al. 1996; Sah and McLachlan 1995; Yoshimura et al. 1987) and particularly the in depth characterization by Whyment *et al* (2011) and Bordey *et al* (1995) (see Appendix). All active conductances were present in the soma. The leak current ($I_{\text{pas}}$) was present throughout the cell. The axon had the Hodgkin-Huxley conductances required for spike generation ($I_{\text{Na},3}$, $I_{\text{DR}}$). The dendrites were passive.
Model Simulation

Simulations of our single-cell model were performed on a two dual-core Opteron processors 8GB RAM node, using the computational facilities of the Advanced Computing Research Centre, University of Bristol, UK (http://www.bris.ac.uk/acrc/). Simulation data was imported into MATLAB 6.1 (The MathWorks Inc., Natick, MA, 2000) for analysis and graphing on a personal desktop (Toshiba Tecra). Statistical tests were conducted in Prism v2.0 (GraphPad). As well as looking at the output from the model in simulated current- and voltage-clamp modes, it was also driven in a more physiological mode with experimentally recorded synaptic currents to generate spike activity. These data were obtained from 50s voltage-clamp recordings from MVC-like SPN.
Results

To compare the electrophysiological properties of SPN from SH and WKY rats, we obtained whole cell recordings from neurons in the working heart brainstem preparation (Paton 1996). A total of 90 SPN (n=50 WKY, n=40 SH) were recorded from 66 WHBP (32 from SH and 34 from WKY rats). SPN were classified on the basis of their responses to cardiorespiratory reflex activation (Stalbovskiy et al. 2014). We identified the muscle vasoconstrictor-like (MVC\textsubscript{like}) class of SPN by their excitatory responses to peripheral chemoreflex activation and the diving response initiation (Stalbovskiy et al. 2014). The dataset reported included 22 MVC\textsubscript{like} SPN from WKY and 23 MVC\textsubscript{like} SPN from age matched SH rats (postnatal days 12.7±0.5 vs 11.4±0.5, respectively; p=0.7).

**Increased spontaneous activity of MVC\textsubscript{like} SPN in SH rats**

In both strains the MVC\textsubscript{like} spike discharge showed respiratory modulation (Figure 1A, B). However the average firing frequency of MVC\textsubscript{like} SPN in SH rats was 58% higher than WKY (3.85±0.39Hz, n=23 vs 2.44±0.35Hz, n=22, p=0.011, Figure 1C). For each cell, action potential discharge was binned into eight 45 degree bins across the respiratory cycle (WKY n=19, SH=20 SPN, activity averaged across 10 cycles for each cell). This respiratory phase analysis of firing showed that SPN in both strains had a peak of discharge in the late inspiratory (I-) and early post inspiratory (PI) phases (Figure 1D, E; peak at 45° in both strains, one way ANOVA) with a tendency for an earlier start to the inspiratory burst in the SH rat. Comparison of these firing histograms showed that both strain and the phase through the respiratory cycle were significant sources of variation (which show influence of both phase (P<0.0001) and strain (P<0.01) with an interaction P<0.01, two-way mixed measures ANOVA) with a significant increase in the peak seen particularly in the 45° bin in the SH rat (P<0.001, Bonferroni post hoc test). The increased overall firing rate of MVC\textsubscript{like} SPN in SH
rats comprised a potentiation of both the respiratory modulated component and a basal component (Figure 1D, E). The basal level of discharge was increased 1.87-fold in SH rats, from 0.49±0.08 spikes/respiratory cycle in WKY (n=19) to 0.93±0.15 spikes/respiratory cycle in SH rats (n=20; P=0.016). The amplitude of the peak discharge was also increased, from 0.87±0.16 spikes/respiratory cycle (WKY, n=19) to 1.96±0.35 spikes/respiratory cycle (SH, n=20; p<0.0001; peak-to-peak). The degree of respiratory modulation of spike discharge, as measured by the peak-to-trough difference in spike count across the bins for each neuron, was amplified 2.2-fold in the SH rats (WKY 0.73±0.11spikes/respiratory cycle (n=19) vs SH 1.74±0.32spikes/respiratory cycle (n=20); p=0.002; Figure 1F).

Importantly, in considering the origin of this altered respiratory modulation, we found no difference in respiratory rate (0.39±0.04 WKY vs 0.35±0.03Hz SH, p=0.52) or inspiratory duration (539±35 WKY vs 466±36ms SH; p=0.16 (n=31 WKY & n=24 SH preparations)). In both strains peripheral chemoreflex activation (50ul, 0.03% NaCN, n=21 WKY, n=19 SH) produced similar increases in respiratory frequency (0.20±0.03Hz WKY vs 0.16±0.02Hz SH; p=0.39), increased phrenic amplitude (4.24±0.87µV WKY vs 5.05±1.42µV SH; p=0.73) and decreased heart rate (1.19±0.11Hz WKY vs 0.98±0.08Hz SH; p=0.12). However, peripheral chemoreflex activation produced a greater increase in firing in MVC_{like} SPN of SH than WKY rats (4.27±0.81Hz, (n=19) vs 1.37±0.31Hz, (n=21); p=0.0018). Given that respiratory and parasympathetic vagal measures of the magnitude of the chemoreflex are similar across the strains, this elevated firing response suggests altered excitability in the sympathetic vasomotor pathway in the SH rat downstream of the respiratory pattern generation network.

Altered MVC_{like} excitability in the SH rat

Given the increased firing activity of MVC_{like} SPN in the WHBP from the hypertensive strain we tested whether this was related to differences in their intrinsic electrophysiological properties? Comparison of MVC_{like} SPN between WKY and SH rats
(Table 1) showed that they have similar resting membrane potentials and action potential threshold, amplitude and duration. Interestingly, given the increased ongoing firing frequency, the after-hyperpolarization (AHP) was both smaller and shorter in the SH than the WKY (14.6±0.3 vs 17.7±0.7mV, p=0.0002; 120.8±10.8 vs 179.6±19.5ms, p=0.011; Figure 2). In addition the input resistance of MVC<sub>like</sub> SPN was greater in the WKY (446±51MΩ; n=18 vs SH 307±33 MΩ; n=16; p=0.033). Thus, the intrinsic properties of SPN differ between SH and WKY rats.

**Enhanced excitability and output in the SH is specific to MVC<sub>like</sub> SPN**

To test whether these changes were generalizable to other classes of SPN we also analyzed data from cutaneous vasoconstrictor-like (CVC<sub>like</sub>) SPNs as a comparator cell class that were characteristically inhibited by both peripheral chemoreflex and diving response (WKY n=15, SH n=6, (Stalbovskiy et al. 2014)). The CVC<sub>like</sub> SPN showed no significant differences in their firing rate or intrinsic properties between SH and WKY rats (Table 2), suggesting that the changes in excitability are specific to the MVC class.

**MVC<sub>like</sub> SPN in the SH rat have reduced transient rectification**

We noted additional differences in the membrane potential responses of MVC<sub>like</sub> SPN to current injection across strains that suggested an alteration in the transient rectification in the SH rat. Specifically the recovery trajectory of the membrane potential from a hyperpolarized level (after current pulse injection) showed a clear inflection point on repolarization followed by a delayed return to rest in all of the WKY MVC<sub>like</sub> SPN (n=18, Figure 3A). This inflection is a well-characterized consequence of activation of an A-current (I<sub>A</sub>) (Miyazaki et al. 1996; Pickering et al. 1991; Sah and McLachlan 1995; Whyment et al. 2011; Wilson et al. 2002; Zimmerman and Hochman 2010) and has been noted previously as a distinct feature of the MVC<sub>like</sub> SPN (Stalbovskiy et al. 2014). The majority of MVC<sub>like</sub> SPN...
in SH rats also exhibited an inflection (71%, 12/17) but it was markedly less pronounced than in the WKY (Figure 3B). The remainder of SH MVC-like SPN (n=5) did not exhibit any inflection on repolarization, instead showing a passive trajectory. The potential at which the inflection occurred ($V_{RI}$) was measured at the abrupt transition from initial passive exponential repolarization to a shallower, almost linear $dV_m/dt$ (Figure 3A). $V_{RI}$ was shifted to a more depolarized potential in SH rats (WKY -62.4±1.7mV, n=18 vs SH -55.3±1.1mV, n=12; p=0.0043; Figure 3C1). As a consequence of this transient rectification the time-to-first-spike was much longer in WKY compared to SH rats (577±132ms, n=16 vs 213±53ms, n=12; p=0.0076; Figure 3C2). These data suggest a reduction in $I_A$ in the MVC-like neurons of the SH rat.

**Modeling the influence of the A-current on SPN excitability**

Given the known influence of $I_A$ on the excitability of other neurons (Connor and Stevens 1971; Rush and Rinzel 1995), we hypothesized that the alteration in transient rectification in MVC-like SPN of the SH rat may account for the altered repolarization and reduced size of AHP and the increased excitability. To test this hypothesis, we constructed a conductance-based compartmental model of the SPN in the WKY (see Computational Overview, Figure 4 and Appendix). We systematically adjusted the parameters of the model $I_A$ to generate a biophysically accurate recapitulation of SPN transient rectifier kinetics in *vitro* (Bordey et al. 1995; Sah and McLachlan 1995; Whyment et al. 2011). The characteristics of the conductance closely matched the experimentally derived values (see Appendix).

The electrophysiological characteristics of the SPN model both qualitatively and quantitatively resembled the experimental recordings of MVC-like SPN in WKY (Figure 4, Table 3). The model produced action potential firing after an initial delay in response to depolarizing current injection (Figure 4D). It also shows an inflection and delayed return to
rest on repolarization after the injection of a hyperpolarizing current pulse (Figure 4D). The action potential waveform of our model cell was similar to the WKY MVC-like SPN with similar threshold, amplitude, duration and AHP morphology (Figure 4E, Table 3). Likewise the current - firing frequency relationship of the model was within the observed range for WKY MVC-like SPN (Figure 4F).

**Relationship between \( I_A \) and model excitability**

Having established that the model recapitulated many of the features of the WKY SPN we systematically examined how the A-current could influence excitability. We found that perturbation of the \( I_A \) conductance parameters (maximal conductance density \( \bar{g}_A \), activation parameters \( V_{1/2}^n \) (half-activation voltage) and \( \zeta_n \) (slope parameters) and the equivalent inactivation parameters \( V_{1/2}^l \) and \( \zeta_l \) were all able to alter the VRI, AHP amplitude, AHP duration and firing frequency of the model. However, only \( \bar{g}_A \) altered those features in a manner consistent with the experimental data for MVC-like SPN in SH rats (Figure 5). In contrast, the other A-current parameters that were tested exhibited contrary effects on the excitability and output of the model (see Appendix). For example both of the inactivation parameters \( V_{1/2}^l \) and \( \zeta_l \) had opposing effects on VRI and firing frequency. The activation parameters \( V_{1/2}^n \) and \( \zeta_n \) both shifted VRI in a depolarizing direction and increased the firing frequency, however the AHP became larger (unlike that seen in the SH rat recordings).

On this basis we focused on the influence of \( \bar{g}_A \) on cell excitability. Reduction of \( \bar{g}_A \) from 12mS/cm\(^2\) to 6mS/cm\(^2\) caused a depolarizing shifted in VRI of 5mV (Figure 5A1). A 74% reduction in \( \bar{g}_A \) recapitulated the experimentally observed difference in VRI between strains (WKY -62.4±1.7mV to SH -55.3±1.1mV, Figure 5A2). The action potential AHP morphology was also sensitive to variation of \( \bar{g}_A \) (Figure 5B) with amplitude (Figure 5C) and duration (Figure 5D) decreased in proportion to \( \bar{g}_A \) (spike discharge driven in the model by
EPSC input shown in Figure 6). In response to a reduction in $g_A$ of 33.8% and 27.3% the model AHP amplitude and duration (respectively) changed from the experimental WKY to the SH data (Figure 5C, D). Consistent with our experimental comparison of SPN from SH and WKY rats, these changes in $g_A$ had comparatively little influence on resting membrane potential, and action potential threshold, amplitude or duration (Figure 5B).

Response of the model SPN to EPSPs is enhanced by reduction of $g_A$

To examine the influence of $I_A$ on the pattern of firing activity we drove the model with experimentally recorded synaptic currents from a WKY MVC-like SPN (Figure 6). Decreasing $g_A$ by 25% from 8mS/cm² to 6mS/cm² increased the action potential response to the same synaptic drive by ~3Hz (Figure 6A, B respectively). Examination of the $g_A$ - firing frequency response curve showed that reducing $g_A$ by 12.7% (from 7.47 to 6.52mS/cm²) was sufficient to increase the average firing frequency from the WKY to the SH values for MVC-like SPN (Figure 6C). Thus, relatively small alterations to $g_A$ can induce a marked increase in the firing frequency response to a given synaptic drive.

Reducing $g_A$ increases sympathetic-respiratory coupling

We next queried whether such reductions $g_A$ could account for differences in sympathetic output across the respiratory cycle between strains (Figure 6A,B). Simulations with control and reduced $g_A$ both show a clear respiratory modulation of spiking reflecting the pattern of the underlying synaptic drive. The peaks in firing occurred during the I-/PI-phase with troughs during expiration. As $g_A$ was varied, the peak-to-trough difference in spike count followed a linear relationship (Figure 6D), indicating that a reduction in the $I_A$ current density amplifies the degree of respiratory-sympathetic coupling in a manner consistent with the data for SH rats (cf Figure 1).
As $\tilde{g}_A$ is varied the patterns of spike output from the model in response to the same experimentally recorded train of EPSCs closely resembled the patterns of activity of SPN from WKY and SH rats (Figure 7A-D). We sought evidence for this effect of the transient rectification *in vivo* by plotting $V_{RI}$ (a measure of the strength of A-current) against the firing frequency for WKY (n=18) and SH (n=12) MVC-like SPN (Figure 7E). This shows a continuum of values across strains with a trend towards higher firing frequencies with more depolarized values of $V_{RI}$. This was clearly seen in the WKY population with a correlation between depolarized $V_{RI}$ (less A-current) and firing frequency; the linear regression revealed that the strength of the transient rectification accounted for ~35% of the variance in firing frequency seen across neurons.

**$I_A$ regulates excitability by an action on synaptic integration**

To begin to explore the mechanism by which reduction in $I_A$ increases action potential output we probed the influence of $\tilde{g}_A$ on the number of underlying threshold crossing synaptic events (with sodium spiking inactivated) compared to the number of action potentials discharged under control conditions (Figure 7F). The event counts (suprathreshold EPSPs and action potentials) under each condition follow a very similar relationship indicating that it is an increase in the number of threshold crossing events that drives the majority of the change in firing rather than a shortening of the refractory period after an action potential. Note also that changing $\tilde{g}_A$ had relatively little influence on the action potential threshold. These simulations suggested that the increase in output with reductions in $\tilde{g}_A$ is a consequence of altered synaptic integration; however this did not preclude a possible difference in the synaptic drives across the strains.
We analyzed the properties of the synaptic input to the MVC-like SPN to see whether the strains had different synaptic drives. The MVC-like SPN (n=7 WKY, n=6 SH) were voltage-clamped close to rest (V_h=-53mV) to obtain a measure of the frequency, amplitude and respiratory modulation of incoming EPSCs (Figure 8). This synaptic drive displayed respiratory modulation in both strains (Figure 8A1 & A2), with the larger amplitude events (>30pA) clustered during the I- and PI-phases. The mean holding current was not different across the strains (WKY -49.6±12.4pA vs SH -56.6±13pA; p=0.71; Figure 8C). Similarly, there was no difference in the frequency of occurrence of the synaptic events (> 10pA, Figure 8B1) across the strains (WKY 14.6±3.4Hz, compared to 12.7±3.4Hz in SH rats (p=0.71)). There was no difference in the proportional distribution of synaptic amplitudes across the strains (Figure 8B2, two-way ANOVA (Bonferroni post-hoc tests)). We also made an assessment of the respiratory modulated component of the synaptic drive. The synaptic charge transfer (measured as the area under the inspiratory burst) of the respiratory modulated component tended to be smaller in WKY (-6.0±2.4pC vs -20.3±7.0pC, p=0.104; Figure 8D). Therefore, based on this sample of voltage clamp recordings, we were unable to demonstrate significant differences in the synaptic input to MVC-like SPN across the strains of rat despite there being clear difference in firing frequency under current clamp conditions.

**Influence of \( \tilde{g}_A \) on model response to EPSCs from MVC-like SPN across strains**

Given that there was a trend towards altered respiratory modulation of the synaptic drive to the MVC SPNs in the SH rat we examined the influence of \( \tilde{g}_A \) on model SPN firing frequency (Figure 8E) when driven with synaptic inputs from MVC-like SPN from WKY and SH rats (n=2 each strain, at each end of the range of amplitudes). The average firing frequency produced by the model in response to these inputs, and its dependence on \( \tilde{g}_A \).
followed a similar profile, irrespective of the source of the input across the SH and WKY strains (Figure 8E).

I_A regulates the decay of EPSPs (and hence summation) in SPN

How do variations in \( g_A \) lead to differences in synaptic integration and output of the model? To address this question we examined the influence of I_A on the sub-threshold summation of EPSPs (Figure 9). The model SPN was challenged with a synthetic synaptic input, to mimic a typical EPSP in SPN (Spanswick et al. 1998). The rate of decay of the resultant EPSP increased with \( g_A \) (with a time constant of 25ms at 6mS/cm^2 to 17.5ms at 12mS/cm^2, Figure 9A2), but the EPSP amplitude was only minimally affected (<5% over the same range of \( g_A \), Figure 9A3). To explore the influence of this change in EPSP decay on summation, we generated pairs of identical EPSCs that were played into the model at varying intervals across a range of \( g_A \) values (Figure 9B1, high \( g_A \) - 12mS/cm^2 and low \( g_A \) - 6mS/cm^2). The gain index was measured as the summated amplitude of the second pulse, as a ratio of the single pulse height (Figure 9B2). This showed that I_A acts to decrease the summation of EPSPs incoming in the frequency range between 15 and 40Hz (Figure 9B) effectively imposing a low pass filter characteristic on the SPN output.

We examined how this influence of \( g_A \) on synaptic integration could shape the sympathetic output using a more physiological input of the experimentally recorded EPSC train over a respiratory cycle (Figure 9C). The model generated progressively more action potentials from the high-frequency synaptic events incoming during the I/II-phase as \( g_A \) was reduced and the respiratory-modulated burst emerged earlier in the I-phase. This low pass filtering property of the A-current therefore influences the pattern of firing of MVC-like SPN by attenuating summation particularly during the respiratory barrage of synaptic inputs.

Diminution of I_A – as seen in the SH rat – thus has a profound effect on the spiking output through a failure of the low pass filtering action on the incoming synaptic drive.
Blocking $I_A$ with intrathecal 4-AP increases SNA and Traube-Hering wave amplitude

To test the principle that the A-current is acting to filter and regulate the sympathetic outflow we recorded thoracic SNA in DAPR of WKY rats (n=5) and examined the response to an intrathecal bolus (100nM in 2-10ul) of the potassium channel blocker 4-aminopyridine (4-AP, Figure 10). Both SNA and perfusion pressure increased in response to the bolus (Figure 10A). Thoracic SNA was significantly increased by 4-AP (Figure 10B; baseline, $37.7\pm10.2\mu V; 4$-AP, $49.7\pm13.5\mu V; p=0.03$). The amplitude of Traube-Hering waves - measured as the peak-to-trough change in perfusion pressure - was more than doubled after the application of 4-AP (Figure 10C; baseline $0.37\pm0.15$ vs 4-AP $0.99\pm0.26$ mmHg; p=0.01). These findings are consistent with the A-current playing a role in governing the sympathetic outflow at a spinal level and its blockade increases both the sympathetic outflow and its consequent vasoconstrictor action on the vasculature.
Discussion

In this investigation we obtained whole cell recordings from MVC\textsubscript{like} SPN in SH and WKY rats \textit{in situ} to identify differences in the integrative properties and synaptic drive in the hypertensive strain that are present before the development of overt hypertension. We find that the SH rat MVC\textsubscript{like} SPN have a 1.6 fold higher frequency of action potential discharge with a greater degree of respiratory modulation of their firing than MVC\textsubscript{like} SPN in WKY rats (no difference across strains was noted in the CVC\textsubscript{like} SPN in any parameter). This increase in SH MVC\textsubscript{like} discharge was associated with a smaller and shorter AHP accompanied by signs of a weaker transient rectification.

This led us to examine the hypothesis that the increased firing in MVC\textsubscript{like} SPN in the SH rat was due to reduced I\textsubscript{A}. We explored this hypothesis by constructing a mathematical model of MVC\textsubscript{like} SPN in the NEURON environment (Hines et al. 2004) with a high fidelity reconstruction of I\textsubscript{A} based on experimentally derived values (Bordey et al. 1995; Sah and McLachlan 1995; Whyment et al. 2011). Besides recapitulating many of the characteristic intrinsic features of SPN noted from experimental studies, this model produced the anticipated patterns of action potential discharge when challenged with synaptic drives recorded from SPN \textit{in situ}. The model was found to be particularly sensitive to variation of the maximal conductance density ($\bar{g}_A$); for example, a 12.7\% reduction could change the action potential discharge frequency of the model from a WKY to a SH rat characteristic and also increased the model SPNs intrinsic excitability in a manner consistent with that seen experimentally. On the basis of this modeling data we propose that the pattern of increased sympathetic activity in the SH rat could be adequately explained by the reduction in I\textsubscript{A}.

Consistent with this proposition we found that intrathecal administration of 4-AP in the DAPR preparation (Pickering et al. 2006; Sadanada et al. 2011), to block the I\textsubscript{A} at a spinal level, produced a striking increase in sympathetic nerve activity, accompanied by increased
vascular resistance and greater amplitude of Traube-Hering waves – alterations similar to that reported for the SH rat at this age (Simms et al. 2009). Given that these changes in $I_A$ predate the overt development of hypertension in the SH rat we speculate that they may be causal to, rather than consequential upon, the development of hypertension.

Characteristics of $MVC_{like}$ SPN in SH rats

The $MVC_{like}$ SPN of WKY rats exhibited characteristic electrophysiological properties, similar to those reported in vitro (Sah and McLachlan 1995; Spanswick and Logan 1990; Wilson et al. 2002; Yoshimura et al. 1986b) and in situ (Stalbovskiy et al. 2014) and in vivo (Dembowsky et al. 1986). The $MVC_{like}$ SPN in the SH strain were recognizably similar to those previously documented in Wistar rats in that they showed a respiratory modulated pattern of ongoing action potential discharge driven by underlying EPSPs (Stalbovskiy et al. 2014). There was increased firing in the SH rat strain and enhanced respiratory-coupling, mirroring whole-nerve recordings (Simms et al. 2010; Simms et al. 2009) and reinforcing the principle that there are central changes in the processing of the sympathetic outflow in the SH rat (Morrison and Whitehorn 1984; Schramm and Barton 1979; Schramm et al. 1979).

The resting membrane potential in $MVC_{like}$ SPN in the SH rat was not different to that in the WKY. Similarly there was no change in the threshold for action potential discharge or in the spike amplitude, suggesting that the altered excitability was unlikely to be due to alterations in the sodium or calcium conductances. However, we noted that the SH rat had smaller AHPs and also exhibited a depolarizing shift in the repolarization inflection point ($V_{RI}$) followed by an accelerated repolarization to rest. These latter two features are considered to be characteristics of the transient rectification in SPN (Bordey et al. 1995; Dembowsky et al. 1986; Miyazaki et al. 1996; Pickering et al. 1991; Sah and McLachlan 1995; Whymant et al. 2011) and suggested that there may be an alteration in its expression or kinetics in the hypertensive strain. Intriguingly we noted a positive correlation between the
VRI and the baseline firing frequency of the MVC\textsubscript{like} SPN – suggesting that it may play a role in determining the excitability and output of these neurons \textit{in situ}.

\textit{Influence of $I_A$ on MVC\textsubscript{like} SPN model excitability}

Independent variation of $I_A$ parameters could markedly alter the excitability of the SPN model. Reducing $\bar{g}_A$ shifted $V_{RI}$ in a depolarizing direction from a WKY range to that seen in the SH rat and also decreased AHP amplitude and duration, as seen in the SH rat. A similar action has been shown experimentally in SPN \textit{in vitro} where blockade of $I_A$ with 4-AP was seen to markedly reduce AHP duration and amplitude (Wilson et al. 2002). The parameters describing the steady-state kinetics of the conductance were also systematically investigated (including the activation parameters ($V_{n_1^{-1}}$ and $\zeta_n$) and inactivation parameters ($V_{i_1^{-1}}$ and $\zeta_i$)) to see if they could recapitulate the SH data. Although these parameters all influenced model excitability they each produced contrary changes in either $V_{RI}$ or in AHP amplitude and duration. Therefore we parsimoniously identified $\bar{g}_A$ as being the best candidate parameter and used it to probe the influence of $I_A$ on SPN excitability.

\textit{$I_A$ sculpts SPN responses to incoming synaptic drives}

When challenged with experimentally recorded EPSP trains, a reduction of $\bar{g}_A$ increased the action potential output of the SPN model to a range seen in the experimental recordings from SH rats and similarly increased the degree of respiratory coupling. This reduction in $\bar{g}_A$ in the SPN model was therefore sufficient to recapitulate the altered pattern of output and respiratory-modulation of MVC\textsubscript{like} SPN in SH rats. We also noted that reduction of $\bar{g}_A$ shifted the phase of the start of the respiratory related burst of firing to occur earlier in inspiration, accounting for a phenomenon noted in previous studies of sympathetic-respiratory coupling recorded from whole nerves in the SH rat (Czyzyk-Krzeska and Trzebski 1990; Simms et al. 2010; Simms et al. 2009).
**IA tunes the gain of synaptic integration**

We used the model to gain insight into the mechanics of how the A-current could be acting to alter SPN integration and excitability. The A-current in SPN is unusual in that it has a both relatively slow activation and very slow inactivation (in comparison to that found in many mammalian CNS neurons (Jerng et al. 2004)), resulting in a hyperpolarizing current that lasts for many hundreds of milliseconds (Whyment et al. 2011). This prolonged duration makes it particularly suited to influence events in a frequency range that is associated with the respiratory modulation of SPN activity. Our *in silico* experiments reveal that this long lasting IA endows SPN with the ability to apply a low pass filter to barrages of inputs, with high levels of $\bar{g}_A$ allowing only sparse generation of action potentials with each respiratory cycle. As the density of IA is reduced, each high-frequency synaptic barrage produces a larger burst of action potentials.

The modeling also reveals that IA decreases the decay time-constant of EPSPs, without substantial effects on the magnitude of the EPSP (because of its slow activation characteristic). This suppresses high-frequency (>12Hz) summation of EPSP inputs, hence allowing the neuron to only respond to the strongest EPSP trains incoming during respiratory modulation, thus acting as a low-pass filter. A previous experimental and *in silico* study of sympathetic postganglionic neurons reached a similar conclusion showing that IA also acted to speed the decay of nicotinic EPSPs – making summation less likely (Cassell and McLachlan 1986) but it is clear that this role is likely to be even more important in the preganglionic neuron that is actively integrating high frequency synaptic barrages (Stalbovskiy et al. 2014). We hypothesize that this low pass filtering property is attenuated in MVC-like SPN of the SH rat, resulting in the increased summation of synaptic drives and thus greater transmission of high-frequency, respiratory-modulated bursting to the vasculature.
Is the excitatory synaptic drive altered in the SH rat?

An alternative and/or additional mechanism for the alteration in firing frequency of SPN in the SH rat would be through a change in the synaptic drive to the MVC-like SPN (Sved et al. 2003). To our knowledge no intracellular recordings of SPN have been made to date in the hypertensive strain and so our recordings provide a first direct measure of the synaptic drive. The voltage clamp recordings obtained from the MVC-like SPN showed common patterns of input across strains, with a trend towards an elevation in the respiratory-coupled excitatory drive in SH rats, but we found no evidence for a change in the basal rate or amplitude of synaptic events. We also observed that playing these synaptic current traces into the SPN model recapitulated the patterns of action potential discharge (so we have some confidence in their fidelity) and in each case the resulting output was quantitatively sensitive to the maximal current density of $I_A$. It should be noted however that it is challenging to analyze such massed activity into the component synaptic events and we are only able to resolve the larger events above the baseline, therefore there are limits to our ability to discern specific drives. Hence we cannot discount the possibility of an altered descending drive from the brainstem as has been suggested by the recent findings of increased respiratory drive to pre-sympathetic neurons in the SH rat (Moraes et al. 2014). Further studies to selectively manipulate the descending drives to SPN (e.g. (Abbott et al. 2009)) or the use of focal application of excitatory amino acid antagonists (Stalbovskiy et al. 2014) will be required to help resolve this question of whether there is altered strength of specific descending drives.

Loss of transient rectification in MVC-like SPN in SH rats

Studies of splanchnic (Morrison and Whitehorn 1984) and renal/adrenal (Schramm and Chornoboy 1982) sympathetic outflows in SH rats have attributed the increased activity of the sympathetic pathway to changes at a central and indeed spinal level, respectively. The
impact of spinal cord processes upon the response to descending drives has been elegantly
demonstrated in an optogenetic stimulation study of RVLM C1 neurons that showed a
striking attenuation of the sympathetic response to this descending drive when it was repeated
at short intervals (<2 seconds) (Abbott et al. 2009). This potent filtering effect (previously
referred to as the ‘silent period’) was attributed to the intrinsic properties of the SPN and
places a restriction on the magnitude of the response that can be obtained from a brainstem
input. Interestingly, decreases in the sympathetic ‘silent period’ have been reported in young,
pre-hypertensive SH rats, suggesting that the altered excitability may have its origins in
changes to the rectifying properties of SPN (Schramm and Barton 1979). Our in situ and in
silico findings provide a potential explanation for these experimental observations; the
increased intrinsic excitability, recorded in situ and recapitulated in silico by reducing $g_A$, are
equivalent to the reduced silent period in the SPN. An attenuation of $I_A$ could, therefore,
underlie the previously reported hyper-responsiveness (Schramm and Barton 1979).

Regulation of transient rectification in MVC-like SPN in SH rats

Given that there is an underlying heritable basis to the generation of hypertension in
the SH strain (albeit with a genetic complexity (Marques et al. 2010)) it is interesting to
consider whether a mutation in one of the A-current subunits or the regulatory proteins could
underpin the pathology. Such channelopathies underpin a range of neurological disorders
(Kullmann 2010), although to date there are relatively few reports of syndromes consequent
upon loss of the potassium channel genes responsible for the A-current. There has been a
single report of temporal lobe epilepsy associated with a mutation of the Kv4.2 (Singh et al.
2006) but knock out studies have suggested that this produces a modest change in seizure
threshold and cardiac investigations in the same mouse line showed that the phenotype is
relatively benign with no overt cardiovascular pathology perhaps because of compensation
from other potassium channel subunits (Guo et al. 2005). It should also be noted that the
studies of Whyment et al (2011) have suggested that the SPN transient rectification is likely mediated by Kv4.1 and Kv4.3 so we may not expect to find a sympathetic phenotype in the Kv4.2 knock out.

In this context it may be significant that alteration in excitability seen in our study was restricted to MVC-like SPN and did not extend to changes in CVC-like neuronal activity – implying a functional, cell-type selectivity in the deficit, rather than a global phenotype.

While this change in the A-current in the SH rat could still be a manifestation of cell class restricted inherited predisposition or susceptibility (ie affecting MVC-like but not CVC-like) on the basis of specific genetic expression profiles it could also be a consequence of a targeted signaling event results from differential modulation of MVC-like and CVC-like neurones. There are precedents for such induced changes in the long-term regulation of IA; for example in induced temporal lobe epilepsy models there is an increase in excitability due to reductions in the A-current (Bernard et al. 2004). Similarly β-adrenoceptor-mediated elevations of cAMP and activation of downstream kinases has been shown to produce a depolarizing shift in the activation potential of IA in hippocampal CA1 neurons leading to an increase in neuronal excitability (Hoffman and Johnston 1998). The SH rat has been shown to have altered noradrenergic neuronal function in the brainstem which may lead to altered noradrenaline release in the spinal cord (Kasparov and Teschemacher 2008) which could provide a mechanism for descending neuromodulatory regulation of IA (c.f. (Hoffman and Johnston 1998)). This may be relevant to the recently reported increase in sensitivity of the peripheral chemoreflex (McBryde et al. 2013; Moraes et al. 2014) which is proposed to be driving an increase in sympathetic outflow as this could be acting via altered catecholaminergic (or other neuromodulator) signaling to the cord from the RVLM.

Reductions in IA in pre-sympathetic cardiovascular control centers have been reported in induced (rather than genetic) models of hypertension (Belugin and Mifflin 2005; Sonner et
al. 2008) and these changes have been proposed to contribute to the hyper-excitability. Alterations in $I_A$ have also been reported in sympathetic postganglionic neurons from SH rats that show primary increases in the degree of inactivation in the SH strain (increasing excitability), but these are also accompanied by compensatory increases in the maximal conductance density $\tilde{g}_A$ (Robertson and Schofield 1999) which are consequent upon the development of hypertension. These studies all support the principle that the A-current can be altered in central cardiovascular control circuits in models of hypertension.

The importance of altered bursting activity of MVC-like SPN

Previous studies of sympathetic stimulation indicated that grouped stimuli could induce greater contractile responses in mesenteric arteries (Nilsson et al. 1985), suggesting that bursting preferentially regulates vascular resistance. Recent findings in man have shown that vascular conductance responds to bursting SNA and that the vasoconstriction is dependent on burst amplitude and patterning (Fairfax et al. 2013). On this basis we anticipate that the amplification of the respiratory-component of MVC-like SPN activity in the SH rat by a loss of $I_A$, would therefore be expected to increase vascular resistance in excess of that predicted by simple consideration of the increase in tonic firing rate. It should also be noted that recent in silico investigations have shown that populations of neurons transform common inputs to synchronous output with greater fidelity at lower values of $\tilde{g}_A$ (Barreiro et al. 2012). Thus the reduced $\tilde{g}_A$ in SH rats would be expected to produce an enhanced synchrony of respiratory-modulated bursts across the population of MVC-like SPN. The resultant synchrony and amplification of noradrenaline release onto the artery wall, would be expected to also increase the respiratory modulated vasoconstriction (i.e. Traube-Hering waves) and blood pressure in the SH rat. Our experimental finding that spinal administration of 4-AP to the WKY rat produces just such a pattern of effects supports this contention notwithstanding the effects that the antagonist will have had upon other spinal circuits.
Concluding remarks

Our in situ and in silico experiments indicate that the transient rectification of SPN plays a key role in the process of sympathetic integration – acting as a potentially tunable low pass filter whose slow kinetics are suited to regulation of the amplitude of bursting discharges. We provide evidence that dysfunction of this filter may be sufficient to recapitulate the experimental findings from the SH rat, and although this finding does not preclude altered brainstem mechanisms, it does highlight the importance of SPN properties in contributing to the elevated SNA in the pre-hypertensive rat. These changes in MVC-like SPN properties are found before the onset of hypertension (but at a time when the vascular resistance is already beginning to increase (Simms et al. 2009)) and therefore may be causal rather than consequential. These conclusions highlight the key importance of the intrinsic properties of the SPN in shaping the sympathetic output to the vasculature in pathological conditions and identify it as a possible locus for intervention.
Acknowledgements

This study was supported by the British Heart Foundation (Grant PG/06/084, PI - JFR Paton).

LJBB is supported by an EPSRC/BBSRC PhD studentship. AOS and LJBB contributed equally to this study, and are joint first authors. We are grateful for the contribution of Professor Julian FR Paton, for his supervision and support in the conduct of these studies. We also thank Callum Wright at the Advanced Computing Research Centre (Bristol) for his support in running simulations on BlueCrystal. AEP is a Wellcome Trust Senior Clinical Research fellow.
Table 1: Electrophysiological properties of MVC\_like SPN in WKY and SH rats

<table>
<thead>
<tr>
<th>Property</th>
<th>WKY (mean±SEM)</th>
<th>WKY n=</th>
<th>SH (mean±SEM)</th>
<th>SH n=</th>
<th>P=</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency (Hz)</td>
<td>2.44±0.35</td>
<td>22</td>
<td>3.85±0.39</td>
<td>23</td>
<td>0.01</td>
</tr>
<tr>
<td>Resting Potential (mV)</td>
<td>-53.0±1.2</td>
<td>22</td>
<td>-51.5±1.0</td>
<td>23</td>
<td>0.32</td>
</tr>
<tr>
<td>Input resistance (M(\Omega))</td>
<td>446±51</td>
<td>18</td>
<td>307±33</td>
<td>16</td>
<td>0.03</td>
</tr>
<tr>
<td>Time constant (ms)</td>
<td>28.4±3.0</td>
<td>16</td>
<td>40±6.7</td>
<td>15</td>
<td>0.12</td>
</tr>
<tr>
<td>Threshold (mV)</td>
<td>-42.8±1.0</td>
<td>22</td>
<td>-42.9±1.0</td>
<td>23</td>
<td>0.97</td>
</tr>
<tr>
<td>Spike amplitude (mV)</td>
<td>47.4±2.1</td>
<td>22</td>
<td>47.6±2.1</td>
<td>23</td>
<td>0.97</td>
</tr>
<tr>
<td>Spike duration (ms)</td>
<td>3.40±0.24</td>
<td>22</td>
<td>3.50±0.22</td>
<td>23</td>
<td>0.99</td>
</tr>
<tr>
<td>AHP amplitude (mV)</td>
<td>17.7±0.71</td>
<td>22</td>
<td>14.6±0.34</td>
<td>23</td>
<td>0.0002</td>
</tr>
<tr>
<td>AHP duration (ms)</td>
<td>179.6±19.5</td>
<td>22</td>
<td>120.8±10.8</td>
<td>23</td>
<td>0.01</td>
</tr>
<tr>
<td>Property</td>
<td>WKY (mean±SEM)</td>
<td>WKY n=</td>
<td>SH (mean±SEM)</td>
<td>SH n=</td>
<td>P=</td>
</tr>
<tr>
<td>--------------------------</td>
<td>----------------</td>
<td>--------</td>
<td>----------------</td>
<td>--------</td>
<td>----</td>
</tr>
<tr>
<td>Frequency (Hz)</td>
<td>1.45±0.21</td>
<td>15</td>
<td>1.60±0.61</td>
<td>6</td>
<td>0.73</td>
</tr>
<tr>
<td>Resting Potential (mV)</td>
<td>-51.81±1.06</td>
<td>15</td>
<td>-54.30±2.68</td>
<td>6</td>
<td>0.36</td>
</tr>
<tr>
<td>Input resistance (MΩ)</td>
<td>304.9±63.90</td>
<td>11</td>
<td>301.2±44.60</td>
<td>5</td>
<td>0.65</td>
</tr>
<tr>
<td>Time constant (ms)</td>
<td>26.60±5.72</td>
<td>10</td>
<td>21.00±6.66</td>
<td>5</td>
<td>0.69</td>
</tr>
<tr>
<td>Threshold (mV)</td>
<td>-42.25±0.95</td>
<td>15</td>
<td>-45.95±2.52</td>
<td>6</td>
<td>0.17</td>
</tr>
<tr>
<td>Spike amplitude (mV)</td>
<td>49.93±2.75</td>
<td>15</td>
<td>51.00±4.89</td>
<td>6</td>
<td>0.73</td>
</tr>
<tr>
<td>Spike duration (ms)</td>
<td>3.28±0.20</td>
<td>15</td>
<td>2.91±0.58</td>
<td>6</td>
<td>0.31</td>
</tr>
<tr>
<td>AHP amplitude (mV)</td>
<td>-18.56±1.06</td>
<td>15</td>
<td>-17.33±1.30</td>
<td>6</td>
<td>0.51</td>
</tr>
<tr>
<td>AHP duration (ms)</td>
<td>297.1±36.16</td>
<td>15</td>
<td>288.2±99.71</td>
<td>6</td>
<td>0.37</td>
</tr>
</tbody>
</table>
Table 3 Electrophysiology of model SPN compared to WKY MVC-like SPN

<table>
<thead>
<tr>
<th>Property</th>
<th>Model SPN</th>
<th>SPN in situ (mean±SEM)</th>
<th></th>
<th>Model – WKY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting Potential (mV)</td>
<td>-55.0</td>
<td>-53.0±1.2</td>
<td></td>
<td>2.0</td>
</tr>
<tr>
<td>Input resistance (MΩ)</td>
<td>350</td>
<td>446±51</td>
<td></td>
<td>96</td>
</tr>
<tr>
<td>Time constant (ms)</td>
<td>41.5</td>
<td>28.4±3.0</td>
<td></td>
<td>13.1</td>
</tr>
<tr>
<td>Threshold (mV)</td>
<td>-39.1</td>
<td>-42.8±1.0</td>
<td></td>
<td>3.7</td>
</tr>
<tr>
<td>Spike amplitude (mV)</td>
<td>49.5</td>
<td>47.4±2.1</td>
<td></td>
<td>2.1</td>
</tr>
<tr>
<td>Spike duration (ms)</td>
<td>3.0</td>
<td>3.40±0.24</td>
<td></td>
<td>0.4</td>
</tr>
<tr>
<td>AHP amplitude (mV)</td>
<td>17.0</td>
<td>17.7±0.71</td>
<td></td>
<td>0.7</td>
</tr>
<tr>
<td>AHP duration (ms)</td>
<td>175</td>
<td>179.6±19.5</td>
<td></td>
<td>4.6</td>
</tr>
</tbody>
</table>
**Figure Legends**

**Figure 1: Increased activity of MVC\textsubscript{like} SPN in SH rats.**

MVC\textsubscript{like} SPN of WKY (A) and SH rats (B) both exhibited respiratory modulation of discharge entrained to phrenic nerve activity (PNA), but the SH SPN has an increased firing frequency with larger respiratory modulated bursts occurring in the I- and PI-phase. (C) The MVC\textsubscript{like} SPN of the SH rat had a higher mean firing frequency (3.85±0.39Hz, SH (n=23) vs 2.44±0.35Hz, WKY (n=22); p=0.01, t-test). (D, E) Phase histograms of MVC\textsubscript{like} SPN discharge across strains showed a pattern of respiratory modulation of activity (firing activity over the respiratory cycle apportioned into eight 45 degree bins; WKY n=19, SH=20 SPN, activity averaged from 10 respiratory cycles for each cell). The grouped WKY MVC\textsubscript{like} SPN activity had clear respiratory modulation (One-way ANOVA; p=0.002; n=19) with a peak of discharge in the 45º bin (I-phase; p<0.01, ††) compared to the trough during 225º (ME-phase), and also the 135º, 180º and -45/315º bins (p<0.05, †). (E) Similarly, the SH MVC\textsubscript{like} SPN also showed respiratory modulation (One-way ANOVA, n=20, p<0.001) with a peak at 45º when compared to the trough at 135º (p<0.001, †††). The trough now begins 90º earlier (compared to WKY) and the ramp up in activity to the peak starts earlier in the cycle. Comparison across strains showed both strain and phase were significant sources of variation (phase (P<0.0001) and strain (P<0.01) with an interaction P<0.01, two way mixed measures ANOVA) with a significant increase in the peak seen particularly in the 45º bin in the SH rat (P<0.001, Bonferroni post hoc test). Post hoc testing also showed higher basal firing level (shaded) in the SH rats (trough-to-trough, P<0.05, t-test). (F) The degree of respiratory modulation of MVC\textsubscript{like} activity as peak-to-trough difference in spike count across the bins was significantly larger in the SH rats (WKY=0.73±0.11spikes/bin, n=19 vs 1.74±0.32spikes/bin, n=20; p=0.009, t-test).
Figure 2: Reduction in AHP of MVC\textsubscript{like} SPN in SH rats.

(A) Action potential waveforms from a representative WKY (blue) and SH (red) MVC\textsubscript{like} SPN (average of 10 spikes ± SEM) showing reduced AHP amplitude and duration. (B), (C) Across the population the SH rat MVC\textsubscript{like} SPN had shorter (120.8±10.8 (n=23) vs 179.6±19.5ms (n=22); p=0.01), smaller AHPs (14.6±0.34mV (n=23) vs 17.7±0.71mV (n=22); p=0.002).

Figure 3: Diminished transient rectification in MVC\textsubscript{like} SPN of SH rats.

(A) Voltage responses of a MVC\textsubscript{like} SPN of a WKY rat to family of hyperpolarizing current pulses. At the offset of hyperpolarizations greater than -75mV, a clear repolarization inflection point was seen in the decay trajectory (arrow; V\textsubscript{RI}). This signaled the activation of the transient rectification (I\textsubscript{A}) which delayed repolarization and suppressed the firing activity of the cell. (B) V\textsubscript{RI} occurred at a more hyperpolarized level in WKY (B\textsubscript{1}), than the SH rat (B\textsubscript{2}). (C\textsubscript{1}) Grouped V\textsubscript{RI} data (measured on repolarization from a potential of < -75mV) showing it occurred at a more hyperpolarized level in WKY compared to SH rats (WKY=-62.4±1.7mV (n=18); SH=-55.3±1.1mV (n=12); p=0.0043). (C\textsubscript{2}) The time-to-first-spike (measured from the release of the hyperpolarizing current pulse) was shorter in the SH rat (WKY 577±132ms, n=16 vs SH 213±53ms, n=12; p<0.001).

Figure 4: Model of an MVC\textsubscript{like} SPN and comparison of electrophysiology to WKY data.

(A) Schematic morphology of a SPN in the lateral horn of the spinal cord showing its position in the lateral horn, dendritic tree and axon heading toward the ventral root. (B) Morphology of the SPN defined in NEURON with the distribution of ion conductances; the dendrites were passive and the axon had four voltage-dependent conductances ($I_{Na3}$, $I_{Dr}$, $I_{CaL}$...
& I_{CaN}). (C) Soma schematic showing conductances, intracellular buffers and membrane mechanisms. (D) Membrane potential responses of the model to current pulse injections (1s duration). Note the delay to firing (†) and the repolarization inflection (V_{RI}) on return to rest after hyperpolarization (*). (E) Action potential firing was triggered in the model with a small current pulse (5pA). The model spike threshold, AHP amplitude, AHP duration (arrow) and resting membrane potential (RMP) are all within a standard deviation of the experimental data for MVC_{like} SPN in WKY rats (full comparison in Table 3.) (F) The firing frequency of the model to depolarizing current pulse injection fits the experimental data from MVC_{like} SPN in WKY (10pA (2.4±0.6Hz, n=19), 20pA (4.8±2Hz, n=5), 30pA (7.2±1Hz; n=20) and 50pA (10.5±10.9Hz; n=11); response to current pulse injection for 1s).

**Figure 5: Influence of I_{A} on membrane excitability of the model.**

Reducing the maximum conductance density (\(\bar{\bar{g}}_{A}\)) alters the excitability of the model in a manner consistent with the SH data. (A) Step current pulses (1s duration) were injected into the model cell from a potential of -50mV, to hyperpolarize the cell to -100mV to measure \(V_{RI}\). (A1) As \(\bar{\bar{g}}_{A}\) was reduced from 12 to 6mS/cm\(^2\), \(V_{RI}\) shifted in a depolarizing direction (~5mV). (A2) the relationship between \(\bar{\bar{g}}_{A}\) and \(V_{RI}\) showed that reduction of \(\bar{\bar{g}}_{A}\) moved the level of \(V_{RI}\) from the WKY to SH range (shaded regions). (B) The AHP amplitude and duration in the model, was measured from action potential waveforms generated from excitatory input (EPSCs recorded experimentally see Figure 6). Decreasing \(\bar{\bar{g}}_{A}\) from 10.5mS/cm\(^2\) reduced the AHP amplitude (C) and duration (D) from the range of WKY MVC_{like} SPN to values in the range seen in SH rats (shaded regions).

**Figure 6: I_{A} shapes the output of the model.**
The model was challenged with a train of EPSCs (recorded in voltage-clamp (-53mV) from a WKY rat MVC<sub>like</sub> SPN over 100s). The output of the model (firing and pattern) was monitored as the $g_A$ was reduced. (A) For high $g_A$ (8mS/cm<sup>2</sup>), the model produced a low average firing frequency (1.8Hz) with a degree of respiratory modulation (peak-to-trough = 10 spikes), consistent with the WKY data. (B) As $g_A$ was reduced (to 6mS/cm<sup>2</sup>), the average firing frequency (4.7Hz) and degree of respiratory modulation (14spikes) increased, as seen in the SH group. Graded reductions of $g_A$ produced a monotonic increase in the firing frequency (C) and respiratory-coupling (D) of the model, into the ranges seen experimentally across the strains (shaded regions).

**Figure 7:** SPN output characteristic is reconfigured by $g_A$.

The pattern of discharge of the model, in response to a common EPSC drive recorded from a WKY MVC<sub>like</sub> neuron (A top trace), was closely comparable to that of experimentally recorded cells across strains, (A) The frequency and respiratory modulation of firing increased in the model, as $g_A$ was reduced (down the column). (B-D) Experimental recordings of MVC<sub>like</sub> SPN. With higher values of $g_A$ the model exhibited strikingly similar discharge patterns to that seen in WKY MVC<sub>like</sub> SPN (#1) whereas with low values of $g_A$ the model output more closely resembled recordings from SH rats (#3 & #4). (E) Firing frequency of recorded MVC<sub>like</sub> SPN plotted as a function of $V_{RI}$ for WKY (n=18) and SH (n=12). The WKY data was fit with a linear regression ($R^2$=0.35) showing a positive correlation between $V_{RI}$ and the spontaneous firing frequency. (F) Using the model we tested whether the effect of $g_A$ altered the frequency of threshold crossing synaptic events (generated from the EPSC train in A) by inactivating the sodium conductance to prevent action potential discharge. Comparison of the numbers of action potentials with the number of supra-threshold synaptic events revealed a close linkage across $g_A$ indicating that the
major influence of the A-conductance on discharge is through altered synaptic integration rather than by an action on the AHP. We also used the model to investigate the effect of altering $\bar{g}_A$ on the number of underlying threshold crossing synaptic events (with sodium spiking inactivated) versus the number of action potentials discharged to see whether its influence on firing frequency was via an action on synaptic integration or upon the after hyperpolarization (F). The event counts (EPSPs and action potentials) under each condition follow a very similar relationship indicating that it is an increase in the number of threshold crossing events that drives the majority of the change in firing rather than a shortening of the refractory period after an action potential.

Figure 8: Synaptic input to MVC$_{like}$ SPN in WKY and SH rats.

MVC$_{like}$ SPN in WKY (n=7) and SH (n=6) rats were voltage-clamped close to rest (-53mV), to record the spontaneously ongoing excitatory post-synaptic currents (EPSCs). (A) EPSCs incoming to an MVC$_{like}$ SPN in a WKY (A$_1$) and SH rat (A$_2$). Over a 10s period of recording, putative EPSCs were located using a peak-find algorithm (with a rise time of $>1$ms and amplitude $>10$pA in Spike2) (B). The resultant output was manually validated against the raw data, and individual peaks were verified (shown expanded below with event trains). (B$_1$) The frequency of incoming EPSCs of magnitude $>10$pA, was no different across the strains (WKY=14.6±3.4Hz; SH=12.7±3.4Hz; p=0.71). (B$_2$) The proportional amplitude distribution of incoming events was no different across the strains (data from recordings of 6 MVC$_{like}$ SPN per strain, binned into 10-20pA, 20-30pA, 30-40pA or $>40$pA events and expressed as a proportion of the total number of events over a 10s period, two-way ANOVA with Bonferroni post-hoc tests). (C) The mean holding current over the recording was no different across the strains (WKY=-49.6±12.4pA; SH=-56.6±13pA; p=0.71). (D) The magnitude of the respiratory modulated burst of synaptics was quantified by integrating the current during
inspiration. A greater synaptic charge transfer was apparent in the SH rat, but did not reach significance (p=0.104). (E) The model was challenged with EPSC traces taken from each strain (50s duration; 2 WKY (blue), 2 SH (red dashed)). The output discharge of the model, and its relationship to $\bar{g}_A$, was seen to be relatively independent to the strain of origin of the EPSCs.

Figure 9: $I_A$ functions as a low-pass filter of incoming EPSPs.

The influence of $I_A$ on synaptic integration in the model was investigated. (A) Injecting a point synaptic conductance (a double-exponential with rise time of 1.5ms and decay time of 2ms) produced an EPSP, with a profile typical of those recorded in SPN (Spanswick et al. 1998). As $\bar{g}_A$ was reduced from 12mS/cm$^2$ to 6mS/cm$^2$, the time-constant of decay increased considerably (A2) whereas EPSP amplitude increased only marginally (<5%) (A3), this effect is explained by the relatively slow activation of the A-conductance as indicated beneath where the peak conductance change occurs after the peak of the EPSP.

(B) To assess the influence of $\bar{g}_A$ on synaptic summation two EPSPs were delivered at varying intervals (B2). For $\bar{g}_A=6$mS/cm$^2$, the gain of the second pulse (as a ratio of the single pulse height) increased with frequency (B1). For $\bar{g}_A=12$mS/cm$^2$, gain was suppressed (i.e. <1) at high-frequencies (10-45Hz), and was unitary at low frequencies (<10Hz). This suppression of gain is due to the influence of $I_A$ on the decay of the EPSP. (C) An experimentally recorded EPSC train (1 respiratory cycle) was injected into the model, and the output generated was converted into a spike train. For high values of $\bar{g}_A$, the model filters out the synaptics incoming at high-frequency during the inspiratory phase. As $\bar{g}_A$ was reduced, the model transforms more of the high-frequency events during the I-phase into action potentials, and the respiratory-modulated burst increases in amplitude and starts earlier in the cycle.
Figure 10: Intrathecal 4-AP increases tSNA in WKY rats.

Recordings of thoracic sympathetic nerve activity (tSNA) alongside phrenic and perfusion pressure were made from DAPR of WKY rats (n=5). (A) Intrathecal 4-AP (100mM bolus) increased tSNA by blocking spinal I_A. The perfusion pressure was increased due to the increased sympathetic outflow causing vasoconstriction. In addition the respiratory related fluctuation in perfusion pressure (Traube-Hering wave) was also increased in amplitude, as shown in the PNA-triggered average waveforms inset. (B) Mean tSNA in control conditions (37.7±10.2µV) increased (49.7±13.5µV; p=0.03) following 4-AP administration. This highlights the importance of the A-current in regulating spinal sympathetic tone. (C) Traube-Hering wave amplitude (0.37±0.15mmHg) also increased (0.99±0.26mmHg; p=0.01), suggesting amplified respiratory-sympathetic coupling.


Guo W, Jung WE, Marionneau C, Aimond F, Xu H, Yamada KA, Schwarz TL, Demolombe S, and Nerbonne JM. Targeted deletion of Kv4.2 eliminates I(to,f) and results in electrical and molecular remodeling, with no evidence of ventricular hypertrophy or myocardial dysfunction. *Circulation research* 97: 1342-1350, 2005.


Moraes DJ, Machado BH, and Paton JF. Specific Respiratory Neuron Types Have Increased Excitability That Drive Presympathetic Neurones in Neurogenic Hypertension. Hypertension 2014.


Figure 1

A) WKY

B) SH

C) Average Firing Frequency

D) WKY (n=19)

E) SH (n=20)

F) Δ discharge peak-to-trough
Figure 2

A

B

C

AHP duration (ms)

AHP amplitude (mV)

Time (ms)

WKY MVC SPN (mean ± SEM)

SH MVC SPN (mean ± SEM)

WKY

SHR

WKY

SHR

WKY

SHR

***

**
Figure 3

A

B1

B2

C1

C2

$V_{RI}$

Current (pA)

Current (pA)

Current (pA)

Time to first spike (s)

WKY SHR

**

-80

-70

-60

-50

-40

-30

-20

-10

0

WKY

SHR
Figure 4

A  Sympathetic preganglionic

B  NEURON morphology

C  

D  

E  

F  

CA2+ diffusion and buffering

NEURON morphology

Lateral dendrites (200μm x 2μm)

Medial dendrites (600μm x 2μm)

Soma

Axon

IDR  -  Delayed rectifier
INa3  -  Voltage activated Na+ (Nav1.3)
IA    -  Transient rectifier
IAHP  -  Calcium-dependent K+
ICaL  -  Ca2+ - channel L-type
ICaN  -  Ca2+ - channel N-type
IPas  -  Leak conductance
IK, Ca -  Ca2+ & voltage-activated K+

WKY (Mean+/−SEM)  

WKY (Mean+/−SD)

Model

Threshold

AHP amp

AHP dur

RMP

ΔFiring Frequency (Hz)

Current (pA)
**Figure 5**

A1

![Graph A1](image1)

A2

![Graph A2](image2)

B

![Graph B](image3)

C

![Graph C](image4)

D

![Graph D](image5)
Figure 7

A

Model input - (EPSCs from experimental recording in WKY rat)

Model output #2 \( g_A = 9 \text{ mS/cm}^2 \)

Model output #3 \( g_A = 7.5 \text{ mS/cm}^2 \)

Model output #4 \( g_A = 5.5 \text{ mS/cm}^2 \)

B

Experimental recording in WKY rat; MVClike cell #1

C

Experimental recording in SH rat; MVClike cell #2

D

Experimental recording in SH rat; MVClike cell #3

E

WKY data (n=18)

SHR data (n=12)

linear fit to WKY (R\(^2\)=0.35)

F

Action potentials

Suprathreshold events
Figure 8

A1 WKY

A2 SHR

B1 EPSC frequency (Hz)

B2 % of total EPSC events

C Holding current (pA)

D EPSC burst Area (pC)

E Firing Frequency (Hz)

WKY (n=6) SHR (n=6)
Figure 9

A1

Time constant of EPSP decay (ms)

\[ g_A = 12 \text{mS/cm}^2 \]

\[ g_A = 6 \text{mS/cm}^2 \]

~80 ms period of suppression of summation

A2

Gain in EPSP Amplitude

\[ \tau_g \text{ (ms/cm}^2 \]

12

A3

B1

Gain in amplitude of second input

Gain in EPSP Amplitude

\[ \text{Inter EPSP interval (ms)} \]

B2

Gain in amplitude of second input

\[ \text{Instantaneous Frequency of incoming EPSP (Hz)} \]

Gain in amplitude of second input

\[ \text{Instantaneous Frequency of incoming EPSP (Hz)} \]

C

Spike train

EPSCs

[0pA, 100pA]

EPSPs

\[ 14 \text{mS/cm}^2 \]

\[ 13 \text{mS/cm}^2 \]

\[ 12 \text{mS/cm}^2 \]

\[ 11 \text{mS/cm}^2 \]

\[ 10 \text{mS/cm}^2 \]

\[ 9 \text{mS/cm}^2 \]

\[ 8 \text{mS/cm}^2 \]

\[ 7 \text{mS/cm}^2 \]

\[ 6 \text{mS/cm}^2 \]

\[ 5 \text{mS/cm}^2 \]

\[ 4 \text{mS/cm}^2 \]

\[ 3 \text{mS/cm}^2 \]

\[ 2 \text{mS/cm}^2 \]

\[ 1 \text{mS/cm}^2 \]

\[ 0 \text{mS/cm}^2 \]

\[ -55 \text{mV} \]

\[ 20 \mu S \]

\[ 20 \text{ms} \]

\[ -1 \text{mV} \]

\[ 1 \text{mV} \]

\[ 0 \text{pA} \]

\[ 10 \text{pA} \]

\[ 100 \text{pA} \]

\[ 5 \mu V \]

\[ 1000 \text{ms} \]
Figure 10

A

\[ \dot{J}_{\text{SNA}} \]

\[ 4\text{mmHg} \]

\[ 60 \]

\[ 10\text{uV} \]

\[ 50 \]

Control

4-AP

B

C

\[ \dot{J}_{\text{PNA}} \]

Perfusion pressure

\[ 4\text{mmHg} \]

\[ 60 \]

\[ 0.5\text{mmHg} \]

\[ 0\text{mmHg} \]

\[ 500\text{ms} \]

\[ 61 \]

\[ 72 \]

\[ n=5 \]

\[ n=5 \]

\[ n=5 \]

\[ n=5 \]

\[ ** \]

\[ \text{Mean } \dot{J}_{\text{SNA}} (\mu\text{V}) \]

\[ \text{Mean } \dot{J}_{\text{PNA}} (\mu\text{V}) \]

\[ \text{TH Wave Amp (mmHg)} \]