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Sub-Chronic Peroneal Nerve Stimulation Lowers Ambulatory Blood Pressure in Spontaneously Hypertensive Rats

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ABSTRACT *Objective:* Acute electrical stimulation of the common peroneal nerve (cPNS) has been shown to cause an immediate reduction in systolic blood pressure (SBP) in spontaneous hypertense rats (SHR), but the effect of this treatment in sub-chronic ambulatory SBP is unknown. Here we developed an implantable wireless WNClip neural stimulator to test the efficacy of 5-week cPNS as a treatment for hypertension. *Results:* Daily cPNS 2 Hz monophasic stimulation at threshold for 8 minutes every day for five weeks, reduced SBP in WKY animals by -4 mm Hg, and in SHR animals by -21 mmHg in week 5 ($p < 0.01$). Ambulatory SBP measured daily recorded approximately twenty-four hours after the cPNS treatment, showed a significant reduction from the first (176.6 ± 24.1 mm Hg; $n = 5$) to the last week of treatment (165.7 ± 42.7 mm Hg; $n = 4$), a -9 mm Hg reduction ($p < 0.01$). Evaluation of heart rate during the treatment showed no significant difference caused by the daily 8-minute cPNS. *Conclusions:* Electrical stimulation of the common peroneal nerve induced a reduction in SBP that is comparable to that reportedly achieved pharmacologically by ACE inhibitor Ramipril, or by renal denervation procedures. These results support the notion that neuromodulation of the common peroneal nerve can serve as an alternative treatment for drug resistant hypertension.

INDEX TERMS Neuromodulation, resistant hypertension, wireless stimulator, depressor response.

IMPACT STATEMENT This study demonstrates that sub-chronic somatic neuromodulation using an implanted neural stimulator can be used to reduce ambulatory systolic blood pressure in hypertensive animals.

I. INTRODUCTION

Hypertension remains a major health problem characterized by systolic blood pressure (SBP) >130 mmHg and diastolic blood pressure (DBP) >90 mmHg. This condition affects more than 103.3 million people in the USA and is a major contributing factor in the development of cardiovascular diseases such as stroke and heart failure, and kidney failure [1]. Despite current advancements in medical treatments for hypertension a significant proportion of affected patients fail to adhere to their recommended treatments [2], or do not respond to first line medical therapy including combinations of several antihypertensive drugs [3] Drug-Resistant hypertension (RH) affects 15% of hypertensive patients despite full adherence to three antihypertensive drugs of complementary mechanisms including a diuretic agent [4], [5] at maximal doses even with dietary sodium restrictions [6], [7] or

dietary recommendations [8]. RH is associated with a higher risk of cardiovascular disease [9]. Therapy options for RH include the development of new pharmacological agents including a dual endothelin A and B receptor antagonist albeit with modest results [10]. Alternatively, surgical renal denervation aimed at reducing the high sympathetic drive in RH animals [11], has shown to reduce systolic blood pressure (SBP) −5.53 mmHg [12], [13]. However, high variability in the procedure and spontaneous regeneration of the renal nerve limits this alternative [14]. Other treatment options include unilateral resection of the carotid body, which in a cohort of 15 RH patients was shown to reduce ambulatory BP in almost half of the subjects, although serious adverse events were also reported in two of them [15], [16], [17]. Several neuromodulation approaches have been proposed to treat RH [18]. Bilateral electrodes implanted near the aortic arch have been used to stimulate the baroreceptors, which exert an inhibitory influence on sympathetic nerve activity [18]. This approach is currently FDA approved to heart failure in high risk patients and has recently demonstrated to lower BP in patients with RH in a double- blind pivotal trial [19].

Recently, the Rheos (CVRx) system was shown to inhibit sympathetic nerve activity and lower heart rate [15]. However, this neuromodulation therapy failed to reduce ambulatory mean arterial pressure.

We have previously reported that stimulation of the common peroneal nerve induced an acute 15.8 % reduction in SBP values in anesthetized spontaneously hypertensive rats (SHR) [20]. This effect was reproducible and maximal with stimulation parameters that include 1 mA cathodic pulses at low frequency (2 Hz) [21].

We recently developed a fully implantable miniature wireless neural stimulator that allowed sub-chronic evaluation of the effect of common peroneal nerve stimulation (cPNS). Using this strategy, we recently reported that cPNS consistently induced an immediate cardiovascular depressor response that reduces maximal SBP from 132 to 120 mmHg in spontaneously hypertensive rats (SHR) [21]. However, the long-term effect of this therapy and whether it modifies the ambulatory SBP has not been tested.

In this study we evaluate the effect of daily cPNS therapy for up to five weeks to evaluate the effect of this neuromodulation therapy on reducing ambulatory SBP in hypertensive rats.

II. RESULTS

We targeted the common peroneal nerve in the rat hindlimb (Fig. 1(a)), where a wireless neuroclip electrode (wNClip; Fig. 1(b)) was implanted and used as neural stimulation device. The nerve was inserted on the wNClip through a slide-and-lock microchannel (Fig. 1(c)). Two weeks after recovery the animals received wireless stimulation through electromagnetic induction coupling using a near field external antenna with a 6.8 MHz carrier frequency. These animals were also implanted with a blood pressure sensor in the femoral artery for continuous monitoring of SBP using a telemetry system (Fig. 1(d)).

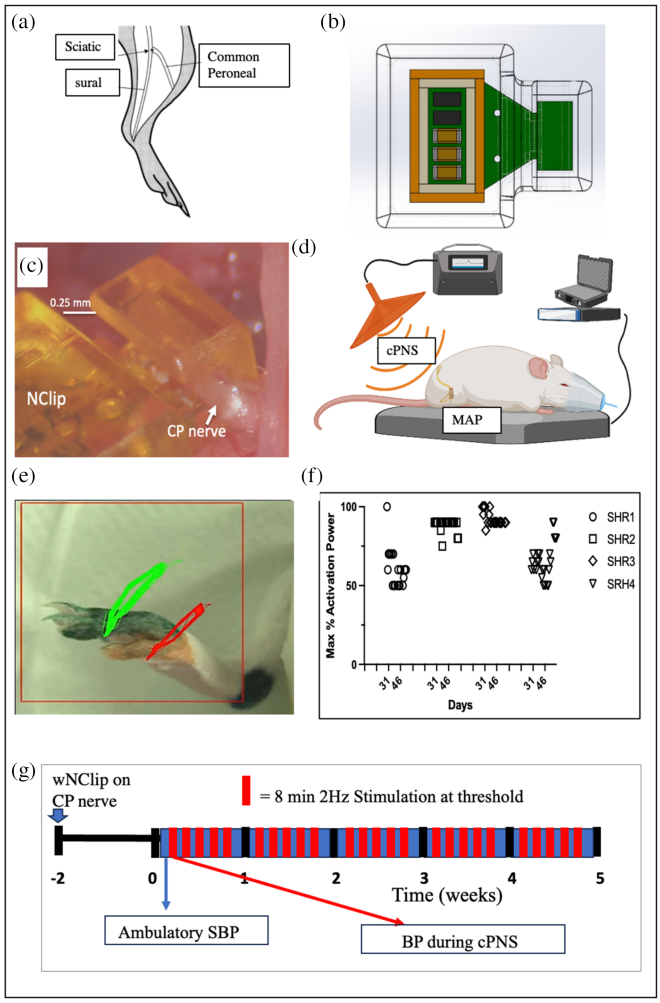

FIGURE 1. Experimental design. (a) Rat hindlimb nerves. (b) Diagram of the wNClip stimulator. (c) Photograph of the device implanted on the common peroneal nerve (cPN). (d) cPN stimulation (cPNS) in anesthetized SHR animals. (e) Color traced limb movement evoked by cPNS. (f) Graph of daily maximum power needed for limb movement threshold (g) experimental design of cPNS (red bars) and continuous BP telemetry during ambulatory (blue bars) and cPNS 5 days a week, for 5 weeks.

At threshold, common peroneal nerve electrical stimulation (cPNS) evokes paw abduction, which was monitored to confirm the function of each implant during the neuromodulation treatment (Fig. 1(e)). The implanted wNClips were reliably activated during the 46 days of implantation and required 50–100% power from the external antenna placed 4–8 cm for inducing limb movement (Fig. 1(f)). This power level has been shown to deliver 100–300 microamps to the nerve, which is above threshold and causes limb movement (Fig. 1(e), (f)). The experimental design is shown in Fig. 1(g). Animals were implanted 2 weeks before the start of the 5-week therapy, thereafter, they received cPNS daily consisting of 8 min of 2 Hz pulsed stimulation at limb movement activation thresholds, 5 days each week. Systolic blood pressure (SBP) was recorded with the telemetry system daily prior to neuromodulation during ambulatory behavior, after anesthesia induction, and during active electrical stimulation (ES) in anesthetized

animals. The effect of cPNS was calculated using the SBP values in the anesthetized animals just prior to stimulation as daily baseline.

A. CPNS REDUCED SBP DURING NEUROMODULATION

The median systolic blood pressure (SBP) values in anesthetized WKY animals in the first week of cPNS treatment was 119.6 ± 22.9 and decreased to 110.9 ± 21.5 mmHg (n = 6) in those same animals during the period of active ES; −7.4% reduction. In comparison, anesthetized SHR animals in the first week, showed median values of 149.7 ± 18.9 mmHg and decreased to 120.3 ± 21.0 mmHg during ES a – 19.6% reduction, twice more than those in the normotensive control group. Fig. 2 shows the total range of SBP values during the 5-week treatment period. One way ANOVA showed a significant treatment effect (F = (316); 7.4; p = 0.003). Tukey's multiple comparisons test showed comparable values in the WKY animals, and significant reduction in anesthetized SHR animals during cPNS (average of 107.4 mmHg) compared to those with only anesthesia (128.4 mmHg) a -16.4% reduction during the treatment period ($p<0.01$), and not significantly different compared to the normotensive controls (105.02 mmHg; Fig. 2(a)).

Heart rate in anesthetized WKY animals averaged 359.4 ± 15.5 BPM (n = 6) in the first week and did not change significantly during ES (335.0 ± 12.1 BPM). Similarly, HR in anesthetized SHR animals was 357.9 ± 33.6 BPM (n = 5) the first week, comparable to those with ES (333.7 ± 46.5), and not statistically different to those in WKY (Fig. 2(b)).

Together, the data showed that cPNS, and not anesthesia alone, lowered SBP in SHR animals, without significantly affecting the HR.

B. AMBULATORY SBP LOWERED BY CPNS IN SHR

Blood pressure was measured daily, approximately 24hrs after the neuromodulation treatments, while animals moved freely. Fig. 3 shows raw data of blood pressure values in a representative SHR sham animal (121-200 mmHg; diastolic-systolic) recorded during 8 min during free ambulation 3 days after implantation (Fig. 3(a)). Similar values obtained in the same animal after 32 days of treatment (136-202 mmHg; diastolic-systolic; Fig. 3(b)). In contrast, values in a representative SHR animal with cPNS showed lower BP values at day 3, (117-180 mmHg; diastolic-systolic; Fig. 4(a)) which further reduced after 39 days of treatment (109-171 mmHg; diastolic-systolic; Fig. 4(b)). This reduction was more evident in the SBP values which are lower than those at d3 (red dotted line in Fig. 4); a -29-mmHg change in SBP due to cPNS in that animal.

Quantitative analysis of ambulatory SBP was done by comparing weekly median SBP values in WKY and SHR animals with cPNS.

The effect of sub-chronic cPNS treatment in SBP is shown in Fig. 5(a). Weekly values in the WKY ES controls did not change significantly due to treatment. Values at first week (133.8 ± 10.1 mm Hg; n = 6) were no different to those at week 5 (141.1± 15 mm Hg; n = 5). In contrast, SHR animals

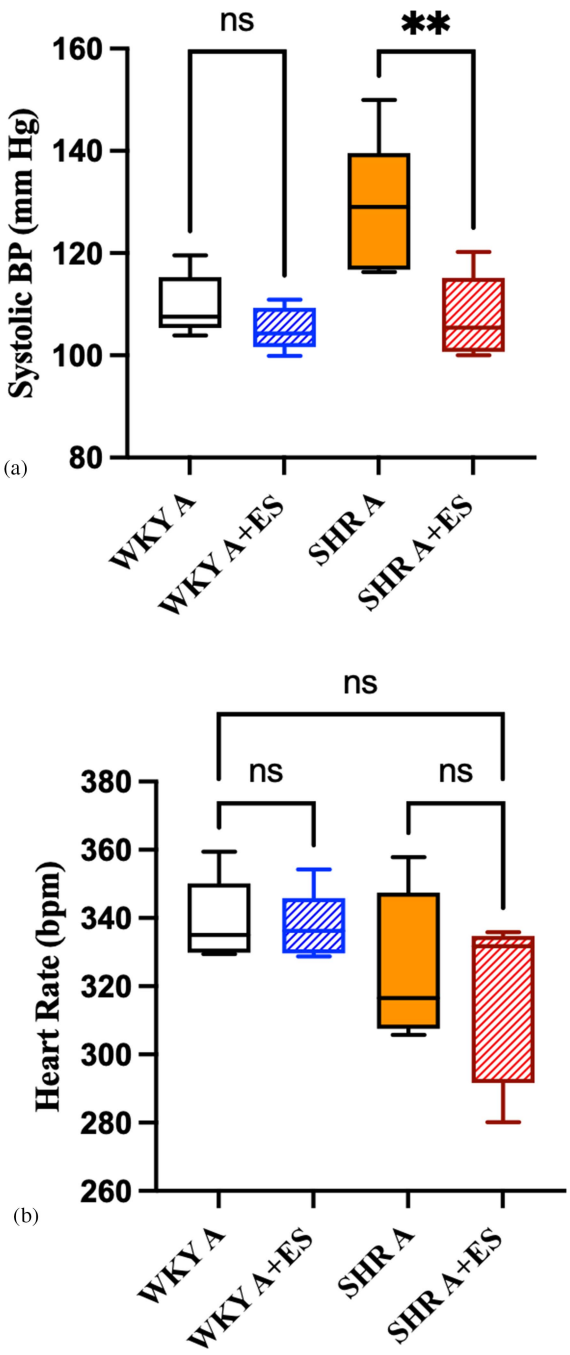

FIGURE 2. Effect of anesthesia (A) and cPNS (ES) on SBP and HR. (a) Neuromodulation did not alter the SBP during ES in WKY animals (n = 6; 28 values), but it reduced SBP in SHR animals (n = 4, 16 values). ∗ = p<0.01. (b) Heart rate was not significantly altered by the treatment.

with cPNS reduced SBP significantly (ANOVA F (12, 38) = 2.1; Fisher's LSD $p < 0.01$) from the first week (176.6 ± 24.1 mm Hg; n = 5) to that of the last week of treatment (165.7 ± 42.7 mm Hg; n = 4), a −9 mm Hg reduction over time. SBP values in cPNS-treated WKY and SHR were comparable at the end of the treatment. These changes were independent of modifications in the HR of these animals since these values did not change significantly between normotensive (292–301

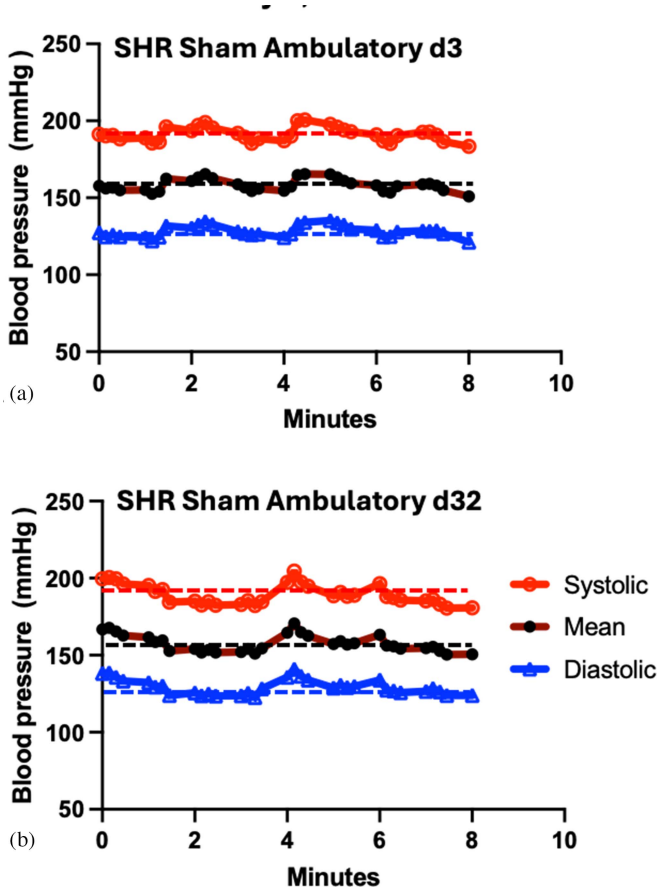

FIGURE 3. Representative BP values in a sham SHR animal. (a) Comparison of BP in the same animal at day 3 (a) and day 32 (b), showed no qualitative change in blood pressure during 8 minutes of free ambulation. The broken line depicts the average values at day 3 for reference.

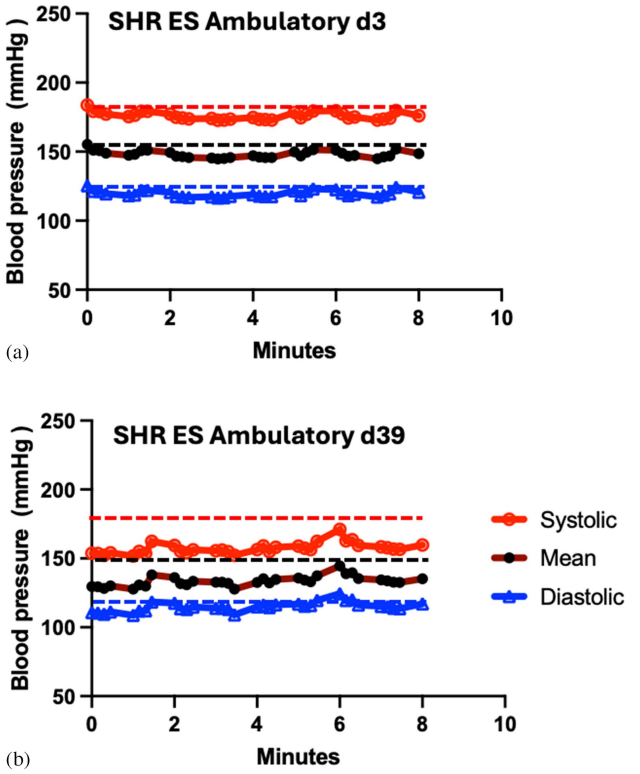

FIGURE 4. Representative BP values in a SHR animals with cPNS. (a) Comparison of BP in the same animal at day 3 (a) and day 39 (b), showed a qualitative reduction in SBP (red dotted line) during 8 minutes of free ambulation. Broken lines depict the average values at day 3 as reference.

BPM) and hypertensive animals (288–306 BPM) for the duration of the study and did not change due to neuromodulation (Fig. 5(b)).

Fig. 6 shows changes in SBP in individual animals due to cPNS. One of the SHR animals did not respond to treatment (N.R.), the other three had different levels of reduction in SBP, with one increasing in week 4, but lowered in week 5. Since the distance of the NClip to the external antenna and the angle was approximated every day for each animal, it is possible that variability in the treatment intensity could explain the differential responses and particularly the increase in one animal at week 4.

III. DISCUSSION

In this study we demonstrated that sub-chronic stimulation of the common peroneal nerve (cPNS) lowered SBP values in SHR animals approximately 20% over 5 weeks of daily neuromodulation therapy is effective, since similar pharmacological studies with the angiotensin-converting enzyme inhibitor Ramipril reportedly lowered systolic BP from 163 to 140 mm Hg; a 15.4% hypotensive effect [22]. Similarly, a 14-day treatment with Ivabradine, a selective blocker of the hyperpolarization-activated cyclic nucleotide–gated channel in SHR rats induced an estimated 5-8% reduction in SBP values [23]. Furthermore, our results seem comparable to renal denervation procedures, as SHR rats undergoing this procedure reduced SBP 19.4% compared to sham-treated controls [13]. Neuromodulation seem to offer an advantage over renal denervation as temporary due to spontaneous peripheral nerves regeneration after injury [14]. However, a direct comparative study will be needed to make that assertion.

The acute hypotensive effect of cPNS has been reported before [21], and is congruent with studies in which 30 min electrical stimulation of the parent Sciatic nerve (0.8-2.0 mA, 0.3 ms pulse duration at 3 Hz) resulted in a -19 mmHg reduction in SBP in pre-hypertensive Dahl sensitive rats [24]. The observed reduction in ambulatory SBP by the 8 min cPNS daily treatment using a fully implantable neural stimulator on the nerve is novel, and relevant to the potential translational value of this bioelectronic therapy alternative for DRH.

Hypertension is a complex condition, and while several factors contribute to its development and progression, overactivity of the renal sympathetic system has been recognized as a crucial factor [25]. Indeed, renal sympathetic nerve activity recorded from the peroneal nerve in patients with kidney failure after bilateral removal, is lower compared to normal subjects [26]. This is consistent with our previous report showing

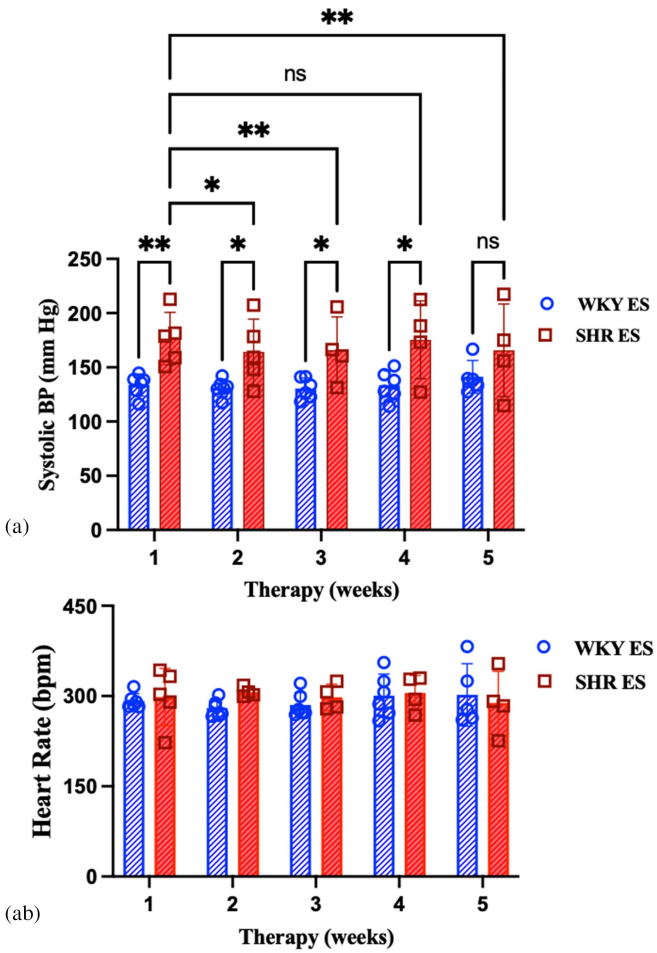

FIGURE 5. cPNS reduces SBP but not HR in SHR animals. (a) Weekly median SBP values in SHR (n = 5) were higher than those at WKY controls (n = 5) at week, but comparable by the last week of treatment). (b) No changes in weekly HR were observed overtime with comparable values in treated WKY and SHR animals. ∗ = p< 0.05; ∗∗ = p<0.01.

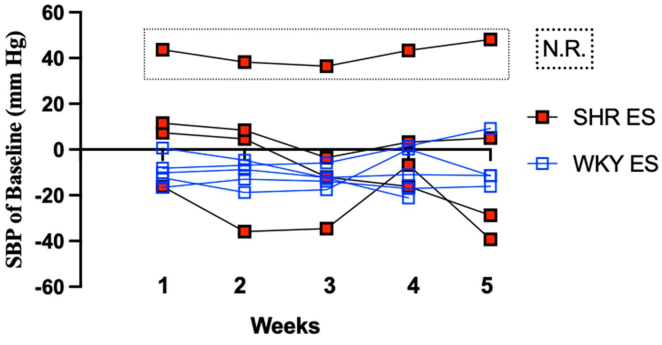

FIGURE 6. Individual ambulatory SBP and response to treatment. Changes in SBP overtime in individual animals shows a non-responder (N.R.) SHR animal, and differential reduction in the other SHR ES animal, with not change or slight increase in the WKY.

that cPNS modulates sympathetic renal nerve activity [21]. It is therefore tempting to associate the observed depressor repones to reduction of the sympathetic nerve activity. However, more studies are needed to fully elucidate the mechanism of action of cPNS on modulating SBP. Other possibilities include the effect of neuromodulation on lowering vascular resistance, since spinal cord epidural stimulation increased femoral vascular conductance (i.e., increase vessel dilation), and reduced muscle sympathetic nerve activity [27]. Clearly, more studies are needed to fully elucidate the mechanism of action of cPNS on modulating SBP in hypertension.

Implantation of the WNClip stimulator directly onto the peroneal neve has the risk of injuring the nerve. This is not supported by our previous studies where we demonstrated that implantation of this neural stimulation directly onto CP nerve, did not show signs of discomfort, or changes in gait, as well as no signs of gross nerve injury or nerve damage or inflammation by immunohistological visualization of myelin and activated macrophages at the site of device implantation [21]. This is also consistent with reports indicating that injury to lower limb nerves increase heart rate and SBP [28], as HR was not modified by cPNS.

Additional work is needed to confirm the long-term effect of this therapy, to define the optimal treatment such as multiple electronic doses per day, and to fully describe the adaptive response of this therapy over time.

The prevalence of hypertension among obese patients ranges from 60–77% in all age groups and it is significantly higher compared to the 34% found in normal weight subjects. In fact, overweight and obese patients represent 15.4% of those treated with antihypertensive medications [29]. This neuromodulation therapy can benefit particularly patients that suffer from obesity or cannot exercise. It is also well known that only a small fraction of these patients will adhere to traditional pharmacological treatments [30], dietary and exercise therapy. Therefore, these patients would be also likely to benefit from this type of neuromodulation therapy

IV. CONCLUSION

In summary, we have demonstrated that neuromodulation of the common peroneal nerve can reduce ambulatory blood pressure and therefore offers a possible therapy for patients suffering from Drug-Resistant Hypertension. Additional work is needed to confirm the long-term effect of this therapy, define the optimal treatment such as multiple electronic doses per day, and fully describe the cellular and molecular mechanisms that explain the adaptive plasticity that seems to mediate the benefit of this therapy over time.

The observed reduction in SBP strongly suggests a process of cardiovascular adaptation. Further studies are needed to develop a better understanding of the mechanistic changes that are elicited by this treatment.

V. MATERIALS AND METHODS

- *Animals:* A total of 15 adult male rats were included in the study divided in Wistar Kyoto (WKY) control animals (n = 6), and spontaneously hypertensive rats (SHR; n = 9; 4 for antenna characterization and 5 for cPNS treatment) (12–14 weeks old 300–350 g; Charles River, MA). SHRs animals are a model of primary hypertension due to an overactive sympathetic drive [31], [32]. Animals were anesthetized with

vaporized isoflurane (2%) in a constant oxygen flux (2 L/min) delivered by a calibrated vaporizer and placed on a warm pad. Body temperature, cardiac rate, and respiration were monitored constantly. The animals were divided into four separate groups. WKY controls, WKY+ES (Electrical Stimulation), SHR control, SHR +ES. This protocol was approved by the Institutional IACUC at the University of Houston (Protocol No. 202000004).

- *wNClip neural stimulator fabrication:* The wNClip are fabricated using UV-sensitive, medical grade, Epo-TEK Epoxy 301, which is non-toxic and ISO 10993 biocompatible, and encapsulated in biocompatible BIO SLA material (USP Class VI). The electrode circuit consists of a copper wire coil for inductive power, connected to ceramic capacitors, microelectronics, and diodes for rectification. The circuit is bonded to gold electrodes which carry the current to the neural interface contact area. Accelerated aging tests were conducted for the wNClip using a 0.9% saline bath at 55 °C (Q = 18, ambient 37 °C) for 32 days, which is equivalent to 1 year in the body. Functional testing for current output was confirmed prior to implantation, with current measured (0.1–0.9 mA) using multimeter at antenna distances 1–5 cm and antenna angles 0–60°. The nerve attachment feature was integrated into the 3D printed nerve-attachment device with a Z-shaped microchannel, leading to a stimulation chamber containing gold trace electrodes. This new design facilitates nerve implantation by placing it underneath the nerve and gently lifting it over the microchannel. This causes a transient longitudinal elongation and transverse compression of the nerve, which then allows it to pass through a smaller microchannel (internal diameter 20–30% less than the CPN diameter) and into the electrode chamber. Lowering the device releases the tension in the nerve. During stimulation we used an external RF antenna. We have shown that electrical fields of ranging from 16.87 to 27.5 A/m, at 4 cm from the implanted wNClip do not exceed the electromagnetic fields limits for safety established by the Federal Communications Commission [33].

- *wNClip implantation:* A 2.0 cm incision on the hindlimb was done to expose the common peroneal nerve branch from the sciatic nerve and gently isolated from surrounding connective tissue using a glass rod and kept hydrated with warm physiological saline solution (pH 7.2). After isolating the CPN, the wNClip was implanted. The wound was then closed after implantation of the wNClip electrode onto the deep peroneal nerve. Topical antibiotics were applied, and prophylactic antibiotic and analgesic were administrated (cefazolin, 5 mg/kg and buprenorphine sustained release], 1 mg/kg; respectively). The animals recovered for 2 weeks under veterinarian supervision before beginning of the wireless CPN neuromodulation sessions.

- *Telemetry Blood Pressure Implantation:* A heparinized (20 IU/ml) pressure catheter was implanted in the femoral artery (0.6 mm OD). BP measurements were obtained continuously. A PowerLab data acquisition system and LabChart Pro software (AD Instruments, Colorado) were used to digitalize and visualize the data. The device's battery was implanted in the abdominal cavity and fixed with sutures to the abdominal walls. An ambient pressure reference (APR-2, DSI) was used for calibration during measurements (accuracy ± 1 mmHg). The measurements were obtained using the PONEMAH software 6.51.

- *Sub-chronic Common Peroneal Nerve Stimulation (cPNS):* Electrical stimulation was delivered in anesthetized animals every day 5-days a week and for 5 weeks. Each daily session was applied in the morning for 8-minutes and consisted of cathodic monophasic pulses of 1 mA in amplitude, and 1 ms pulse duration and at 2 Hz frequency. These stimulation parameters efficiently induced a cardiovascular depressor response as previously reported [21]. The anesthetized animals were placed on a warm pad and placed at a fixed distance (2–3 cm) and angle (0–10^{o}) from the external power antenna. The cPNS stimulation was confirmed by the evoked contraction of the tibialis anterior (TA) muscle which induced the abduction movement of the hindlimb paw, which was used to confirm the threshold and effectiveness of the nerve stimulation, which was found to be reliable for the length of the study.

Systolic blood pressure (SBP) values were recorded during anesthesia and during the active electrical stimulation (ES) of the common peroneal nerve.

- *Ambulatory SBP:* Approximately 24 hours after the cPNS, freely moving animals were kept in their cages where blood pressure and heart rated were measured for at least 10 minutes.

- *Data Analysis.* A custom python code was developed using Spyder version 5.4.1. The DSI data was downloaded in Microsoft Excel format, and python processed heart rate and systolic blood pressure, and separated the times during induction, nose cone, and cPNS for every day and each rat. The program save the SPB and HR data for the 5 minutes before induction, last 2 minutes of induction, last 5 minutes of nose cone, and the entirety of the stimulation time.

- *Statistical Analysis*: The effect of treatment, and animal type was evaluated using Two-way ANOVA followed by an uncorrected Fisher LSD or Tukey's post hoc test. The effect of the sub-chronic stimulation on SBP or HR was evaluated by one-way ANOVA and Tukey's ad hoc test. These tests were performed using the Graphpad Prism software version 10.1.1.

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Conflict of interest: MR-O owns shares in Juniper Biomedical, a medical device company. Juniper Biomedical did not have any role in animal data collection, analysis, or writing the manuscript.

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