Respiratory-gated Auricular Vagal Afferent Nerve Stimulation (RAVANS) effects on autonomic outflow in hypertension*

Roberta Sclocco†, Ronald G. Garcia†, Aileen Gabriel, Norman W. Kettner, Vitaly Napadow, and Riccardo Barbieri, *Senior Member, IEEE*

***Abstract*— Transcutaneous stimulation of the auricular branch of the vagus nerve (ABVN) has been proposed as a non-invasive alternative to vagus nerve stimulation (VNS). However, its cardiovagal effects are inconsistent across studies, likely due to inhomogeneity in the stimulation parameters. Here, we evaluate respiratory-gated ABVN stimulation (Respiratory-gated Auricular Vagal Afferent Nerve Stimulation, RAVANS), where the stimuli are delivered in 1 s bursts during the exhalation phase of respiration, thus mimicking the breathing-induced modulation of cardiac vagal activity. In this study, we present preliminary results from an ongoing single-arm, open label trial investigating the effects of different intensities of RAVANS in hypertensive subjects. We found that a mid-intensity RAVANS stimulation (rated as a 5 on a 0-10 scale) increases the cardiovagal tone and reduces the sympathetic tone during a paced breathing task. The present results could contribute to optimize RAVANS as a non-invasive, low-cost therapeutic intervention for hypertension.**

I. Introduction

Vagus Nerve Stimulation (VNS) is an established treatment for resistant epilepsy or major depression [1]. Traditionally, VNS requires the implantation of a neurostimulating device connected to an electrode located along the cervical branch of the vagus nerve [2]. Recent clinical trials evaluating the use of VNS in patients with heart failure demonstrated a significant increase of parasympathetic tone, as measured by heart rate variability (HRV), and improvement of cardiac structure and function [3], [4]. Despite the reported beneficial effects of VNS, its invasiveness and complications have limited the conduction of clinical trials evaluating its effects on cardiac autonomic modulation for other indications than heart failure. Recently, to overcome some of the adverse events associated with VNS, an alternative non-invasive method of stimulating the vagus nerve has been proposed [5]. This method involves the transcutaneous electrical stimulation of the auricular branch of the vagus nerve (ABVN), which mainly supplies the auricular concha and most of the area around the auditory meatus [6], [7]. Experimental studies have demonstrated that ABVN acupuncture-like stimulation increases parasympathetic tone, reduces arterial blood pressure (ABP) and exerts an antiarrhythmic effect [8]. Clinical studies with electrical stimulation of the ABVN have shown positive effects in vagal activity and cardiac function [9] – [11]. However, others have not found significant effects in heart rate or electrocardiographic parameters [12], [13]. Such varied results might be explained by differences in methodological aspects such as duration or intensity of stimulation.

Animal studies have linked clinical outcomes following ABVN stimulation with medullary activation, in particular of the Nucleus Tractus Solitarii (NTS) [6]. At the same time, respiration can modulate NTS activity. During exhalation, activation of arterial baroreceptors leads to excitation of 2nd-order neurons of the NTS that in turn increase premotor cardiovagal neuron firing rate [14], [15]. In addition, during inhalation NTS receives inhibitory inputs from ventral respiratory nuclei in the medulla [16], [17], reducing vagal outflow to the heart, leading to respiratory sinus arrhythmia (RSA). As the dorsal medullary vagal system operates in tune with respiration, we recently proposed that gating vagal afferent stimulation to the exhalation phase of respiration, would optimize ABVN stimulation and its effects on cardiac vagal modulation, introducing the Respiratory-gated Auricular Vagal Afferent Nerve Stimulation (RAVANS) [13].

In this work, we present the first results from an ongoing study investigating the short-term effects of different intensities of RAVANS on autonomic outflow in hypertensive subjects. In particular, the effects of RAVANS on HRV indices during a paced-breathing task will be evaluated.

II. Materials and Methods

A. Subjects

Twelve (12) subjects (age: 53.2±5.8 years, 7 males) with confirmed hypertension diagnosis as per the 7th report of the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure [18] were enrolled in the study. All subjects were on stable doses of antihypertensive medications at least 30 days prior to entering the study. Exclusion criteria included history or

*Research supported by the American Heart Association (AHA), grant # 16GRNT26420084.

†Authors contributed equally to this manuscript.

R. Sclocco, R.G. Garcia, and V. Napadow are with A.A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA (phone: +1 617 724 1061; e-mails: {roberta, rgarcia, vitaly}@nmr.mgh.harvard.edu).

R. Sclocco, N.W. Kettner, and V. Napadow are with the Department of Radiology, Logan University, Chesterfield, MO, USA (e-mail: Norman.Kettner@logan.edu).

R.G. Garcia and A. Gabriel are with Connor Center for Women's Health and Gender Biology, Division of Women's Health, Brigham and Women's Hospital, Boston, MA, USA (e-mail: agabriel@bwh.harvard.edu).

R. Barbieri is with the Department of Electronics, Information and Bioengineering, Politecnico di Milano, Italy (e-mail: riccardo.barbieri@polimi.it).

R. Barbieri is with the Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA (e-mail: barbieri@neurostat.mit.edu).

evidence of other cardio-, cerebro-, or peripheral vascular disease, diabetes mellitus, obesity, secondary hypertension, kidney or liver failure, history of unexplained fainting spells as self-reported, and current psychiatric or neurological illness. Informed consent was obtained from all participants, and the protocol was approved by the Human Research Committee of Massachusetts General Hospital.

B. *Experimental protocol*

All experiments were performed between 9am and 1pm, in order to control for autonomic variations due to circadian rhythm. Participants underwent 3 sessions on non-consecutive days. In each session, they were asked to perform two non-consecutive 2-minute paced breathing (PB) tasks, and RAVANS was delivered during the second one (RAVANS+PB). Subjects received inhalation and exhalation cues through visual presentation. The paced respiratory cycle had a 5 s duration (2.5 s inhalation, 2.5 s exhalation), in order to be in the physiological range of respiration frequency at rest. Using a fixed respiratory cycle also allowed to control for the amount of stimulation received by each subject.

C. *RAVANS stimulation*

Subjects received RAVANS stimulation in the left ear through ergonomically-shaped Ag/AgCl electrodes (Figure 1). Stimuli consisted of rectangular pulses with 450 μs pulse width and a duration of 1 s, delivered at 25 Hz during each exhalation phase of respiration. The respiratory gating was implemented by feeding the respiratory signal, as measured by a pneumatic belt placed on the chest/abdomen of the subjects, to a laptop-controlled device (National Instruments USB DAQCard 6009, 14 bit i/o, with LabView 7.0 data acquisition software). Computer code provided real-time detection of the end-inhalation peak through an adaptive threshold detection method, and triggered the onset and offset of the stimulation. A 0.8 s delay was introduced between the time of end-inhalation and the beginning of the stimulation, in order to deliver it during the exhalation phase (Figure 1).

The intensity of the stimulation was percept-matched across subjects based on a numerical rating scale (NRS) of 0 to 10 (0 meaning 'no sensation', 10 meaning 'pain threshold'). In two of the three different sessions, current intensity was set to achieve a low intensity ('Low'; NRS target score: 2/10) or a medium intensity ('Medium'; NRS target score: 5/10). In the third session, participants were told that a below-threshold intensity was going to be used, and the stimulus was turned off ('Sham'). The order of the three sessions was counterbalanced across subjects.

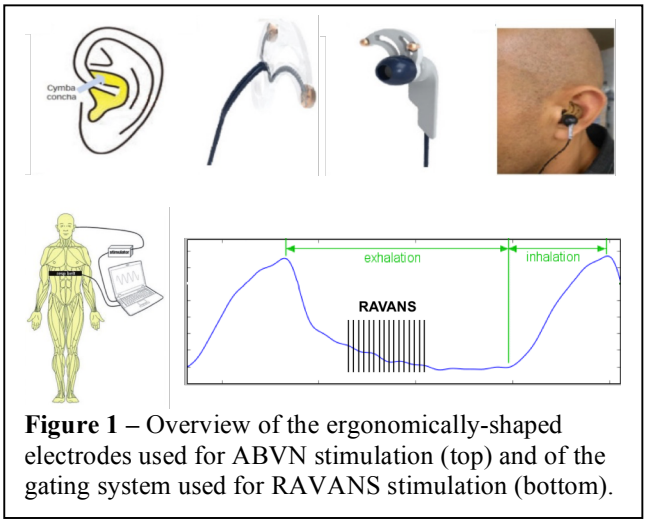

Figure 1 – Overview of the ergonomically-shaped electrodes used for ABVN stimulation (top) and of the gating system used for RAVANS stimulation (bottom).

D. *Data acquisition*

Electrocardiogram (ECG) and respiration were collected continuously at 400 Hz using Chart Data Acquisition Software (ADInstruments, Colorado Springs, CO) on a laptop equipped with a 16-channel Powerlab DAQ System (ADInstruments).

E. *Data analysis*

R-wave peaks from ECG signals were automatically detected using in-house algorithms and visually inspected in order to ensure a correct peak identification. R-R interval series were then fed into a probabilistic model of a dynamical system observed through a point process. In this model, the observation equation summarizes the stochastic properties of the observed heart beat point process, while the essential features of the parasympathetic and sympathetic activity are concisely summarized in a history-dependent, autoregressive time-varying structure [19], [20]. The output of the model were the instantaneous estimates of high and low frequency components of spectral HRV (HF-HRV: 0.15-0.4 Hz; LF-HRV: 0.04-0.15 Hz), that provide a measure of the parasympathetic and sympathetic outflow, respectively. The adopted indices were then evaluated during the PB and RAVANS+PB tasks for each session and subject. In a first within-session analysis, the HF-HRV and LF-HRV difference between the two tasks for the 'Low', 'Medium', and 'Sham' conditions were compared, in order

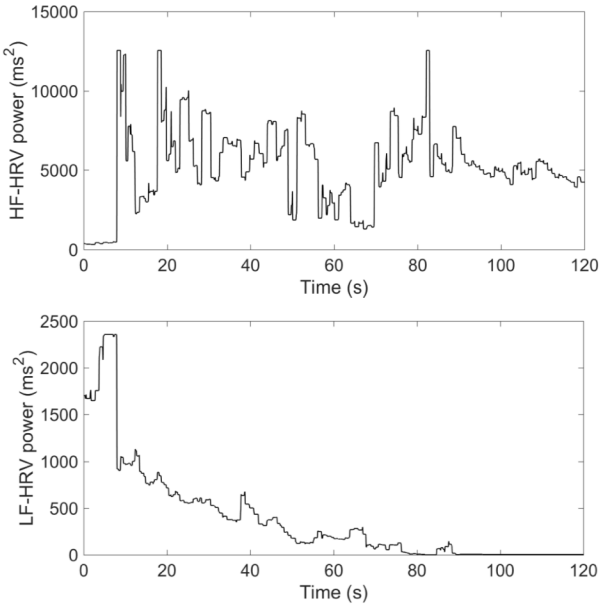

Figure 2 – HF-HRV power (top) and LF-HRV power (bottom) during the RAVANS+PB task for a representative subject.

Table 1 – HF-HRV and LF-HRV power values (mean ± SD) for the within-session analysis (12 subjects, 28 sessions).

		RAVANS + PB		PB	
Sessions	*Intensity*	*HF-HRV power (ms^2)*	*LF-HRV power (ms^2)*	*HF-HRV power (ms^2)*	*LF-HRV power (ms^2)*
9	Low	875.7 ± 1565.1	369.9 ± 301.0	696.6 ± 901.0	508.8 ± 331.4
10	Medium	1611.5 ± 2134.9	548.0 ± 505.7	996.6 ± 1074.0	838.5 ± 1087.7
9	Sham	995.3 ± 1542.1	821.5 ± 559.6	820.4 ± 945.8	807.7 ± 610.2

Table 2 – HF-HRV and LF-HRV power values (mean ± SD) for the across-sessions analysis (6 subjects, 18 sessions).

	RAVANS + PB		
	Sessions	*HF-HRV power (ms^2)*	*LF-HRV power (ms^2)*
Low	6	1164 ± 1836.4	395.8 ± 269.8
Medium	6	1708.3 ± 2035.4	375.4 ± 163.1
Sham	6	1365.9 ± 1775.0	970.5 ± 610.6

to investigate the effect of the stimulation on the autonomic outflow. Subsequently, a subset of subjects (N=6) who completed all three sessions were selected in order to run an across-sessions analysis. HF-HRV and LF-HRV differences between pairs of conditions ('Low-Sham', 'Medium-Sham', 'Medium-Low') during the RAVANS+PB task were evaluated for each subject.

III. Results

All subjects tolerated the stimulation and none of them reported any discomfort due to RAVANS. The current intensities used in the Low and Medium RAVANS sessions were 0.10 ± 0.08 mA and 0.26 ± 0.15 mA, respectively (mean ± SD). In Figure 2, the time-varying HF-HRV and LF-HRV power series during the RAVANS+PB task are shown for a representative subject.

The results from the within-session analysis – that is, the autonomic outflow between the RAVANS + Paced Breathing and the Paced Breathing tasks – are shown in Table 1 and Figure 3A. A general increase in parasympathetic outflow (as measured by HF-HRV power) when paced breathing is paired with RAVANS is present for all conditions, including Sham. The highest increase, however, is found during the Medium RAVANS stimulation (ΔHF-HRV power: 614.9 ± 1249.2 ms^2). As for the sympathetic outflow, LF-HRV power decreases during both Low and Medium RAVANS, with the highest negative variation during Medium RAVANS (ΔLF-HRV power: -290.5 ± 1036.2 ms^2). No visible reduction in sympathetic tone is found during Sham stimulation.

When comparing autonomic changes across sessions during the RAVANS+PB task (Table 1, Figure 3B), the Medium RAVANS condition yields the greatest increase in parasympathetic outflow with respect to both Low (ΔHF-HRV power: 543.9 ± 2294.2 ms^2) and Sham (ΔHF-HRV power: 342.4 ± 1468.2 ms^2). Finally, the sympathetic tone is decreased in both the Low (ΔLF-HRV power: -574.7 ± 691.8 ms^2) and Medium (ΔLF-HRV power: -613.1 ± 492.5 ms^2) RAVANS stimulations compared to Sham.

IV. Discussion

Transcutaneous stimulation of the ABVN has been proposed as a non-invasive alternative to VNS. However, its cardiovagal effects are inconsistent across studies, likely due to inhomogeneity in the stimulation parameters. Here, we evaluate a respiratory-gated ABVN stimulation (RAVANS), where the stimulation is delivered in 1 s bursts during the exhalation phase of respiration. This approach allows to optimize the effect of ABVN stimulation, while also mitigating classical neuronal adaptation/accommodation, which can occur with continuous stimulation of NTS neurons [21].

Importantly, we tested two different intensities of stimulation, percept-matched across subjects, in order to evaluate their effects on the autonomic outflow. With respect to a Sham condition, we found that the Medium intensity (corresponding to a 5 on an NRS of 0 to 10) provided the greatest increases in the cardiovagal outflow, both when comparing the tasks (RAVANS+PB vs PB, Figure 3A) and

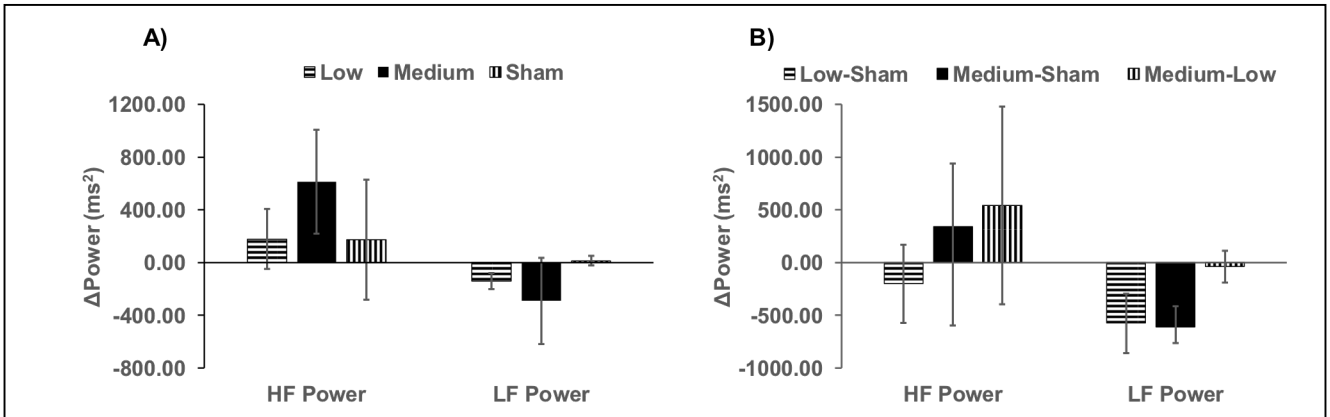

Figure 3 – A) HF-HRV and LF-HRV power difference between the two tasks (RAVANS+PB vs PB; 12 subjects, 28 sessions). B) HF-HRV and LF-HRV power difference between paired conditions during the RAVANS+PB task (6 subjects, 18 sessions). Error bars represent SEM.

the conditions (Medium vs Sham, Medium vs Low, Figure 3B).

While the increase in HF-HRV power was somewhat expected, the concurrent decrease in LF-HRV power during RAVANS suggests effects of this intervention in the modulation of inhibitory interactions between NTS and premotor sympathetic neurons in the brainstem, leading to a general reduction in sympathetic peripheral tone.

The choice of a Paced Breathing task was motivated by the necessity to control for the number of breaths across subjects – delivering a similar number of stimuli – and across tasks – having the same contribution of respiration in the HRV spectral power. In order to evaluate the effects of RAVANS in a condition as physiological as possible, we chose a shorter duration for the respiratory period (5 s) compared to the ones commonly adopted in paced/deep breathing tasks (10 s) [22].

Being this an ongoing study, the sample size is still low, particularly in the across-sessions analysis. No statistical significance was found for the computed indices variations, likely due to the high variance of the indices. However, these preliminary results are encouraging, and the replication of the present analysis on a larger dataset will provide greater power to better characterize the effects of RAVANS of autonomic outflow.

V. Conclusion

In conclusion, our preliminary analysis suggests that mid-intensity RAVANS might be related to a greater short-term modulation of cardiovagal activity in our cohort of subjects. If confirmed in a larger sample size, these results could contribute to optimize RAVANS as a non-invasive, low-cost therapeutic intervention for hypertension.

References

[1] D. J. Englot, E. F. Chang, and K. I. Auguste, "Vagus nerve stimulation for epilepsy: a meta-analysis of efficacy and predictors of response," *J. Neurosurg.*, vol. 115, no. 6, pp. 1248–1255, Dec. 2011.

[2] M. S. George, Z. Nahas, D. E. Bohning, M. Lomarev, S. Denslow, R. Osenbach, and J. C. Ballenger, "Vagus nerve stimulation: a new form of therapeutic brain stimulation," *CNS Spectr.*, vol. 5, no. 11, pp. 43–52, Nov. 2000.

[3] G. M. De Ferrari and P. J. Schwartz, "Vagus nerve stimulation: from pre-clinical to clinical application: challenges and future directions," *Heart Fail. Rev.*, vol. 16, no. 2, pp. 195–203, Mar. 2011.

[4] M. Y. Desai, M. A. Watanabe, A. A. Laddu, and P. J. Hauptman, "Pharmacologic modulation of parasympathetic activity in heart failure," *Heart Fail. Rev.*, vol. 16, no. 2, pp. 179–193, Mar. 2011.

[5] E. C. Ventureyra, "Transcutaneous vagus nerve stimulation for partial onset seizure therapy. A new concept," Childs Nerv. Syst. ChNS Off. *J. Int. Soc. Pediatr. Neurosurg.*, vol. 16, no. 2, pp. 101–102, Feb. 2000.

[6] S. Nomura and N. Mizuno, "Central distribution of primary afferent fibers in the Arnold's nerve (the auricular branch of the vagus nerve): a transganglionic HRP study in the cat," *Brain Res.*, vol. 292, no. 2, pp. 199-205, Feb. 1984.

[7] E. T. Peuker and T. J. Filler, "The nerve supply of the human auricle," *Clin. Anat. N. Y. N*, vol. 15, no. 1, pp. 35–37, Jan. 2002.

[8] X. Y. Gao, Y. H. Li, K. Liu, P. J. Rong, H. Ben, L. Li, B. Zhu, and S. P. Zhang, "Acupuncture-like stimulation at auricular point Heart evokes cardiovascular inhibition via activating the cardiac-related neurons in the nucleus tractus solitarius," *Brain Res.*, vol. 1397, pp. 19–27, Jun. 2011.

[9] A. Zamotrinsky, S. Afanasiev, R. S. Karpov, and A. Cherniavsky, "Effects of electrostimulation of the vagus afferent endings in patients with coronary artery disease," *Coron. Artery Dis.*, vol. 8, no. 8–9, pp. 551–557, Sep. 1997.

[10] A. V. Zamotrinsky, B. Kondratiev, and J. W. de Jong, "Vagal neurostimulation in patients with coronary artery disease," *Auton. Neurosci. Basic Clin.*, vol. 88, no. 1–2, pp. 109–116, Apr. 2001.

[11] R. La Marca, M. Nedeljkovic, L. Yuan, A. Maercker, and U. Elhert, "Effects of auricular electrical stimulation on vagal activity in healthy men: evidence from a three-armed randomized trial," *Clin. Sci. Lond. Engl. 1979*, vol. 118, no. 8, pp. 537–546, Apr. 2010.

[12] P. M. Kreuzer, M. Landgrebe, O. Husser, M. Resch, M. Schecklmann, F. Geisreiter, T. B. Poeppl, S. J. Prasser, G. Hajak, and B. Langguth, "Transcutaneous vagus nerve stimulation: retrospective assessment of cardiac safety in a pilot study," *Front. Psychiatry*, vol. 3, p. 70, 2012.

[13] V. Napadow, R. R. Edwards, C. M. Cahalan, G. Mensing, S. Greenbaum, A. Valovska, A. Li, J. Kim, Y. Maeda, K. Park, and A. D. Wasan, "Evoked pain analgesia in chronic pelvic pain patients using respiratory-gated auricular vagal afferent nerve stimulation," *Pain Med. Malden Mass*, vol. 13, no. 6, pp. 777–789, Jun. 2012.

[14] R. A. Neff, J. Wang, S. Baxi, C. Evans, and D. Mendelowitz, "Respiratory sinus arrhythmia: endogenous activation of nicotinic receptors mediates respiratory modulation of brainstem cardioinhibitory parasympathetic neurons," *Circ Res*, vol. 93, pp. 565-572, 2003.

[15] D. M. Baekey, Y. I. Molkov, J. F. Paton, I. A. Rybak, and T. E. Dick, "Effect of baroreceptor stimulation on the respiratory pattern: insights into respiratory-sympathetic interactions," *Respir Physiol Neurobiol*, vol. 174, nos. 1-2, pp. 135-145, 2010.

[16] M. Miyazaki, A. Arata, I. Tanaka, and K. Ezure, "Activity of rat pump neurons is modulated with central respiratory rhythm," *Neurosci Lett,* vol. 249, no. 1, pp. 61-64, 1998.

[17] M. Miyazaki, I. Tanaka, and K. Ezure, "Excitatory and inhibitory synaptic inputs shape the discharge pattern of pump neurons of the nucleus tractus solitarii in the rat," *Exp Brain Res*, vol. 129, no. 2, pp. 191-200, 1999.

[18] A. V. Chobanian, G. L. Bakris, H. R. Black, W. C. Cushman, L. A. Green, J. L. Izzo Jr, D. W. Jones, B. J. Materson, S. Oparil, J. T. Wright Jr, E. J. Roccella, National Heart, Lung, and Blood Institute Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, and National High Blood Pressure Education Program Coordinating Committee, "The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report," *JAMA J. Am. Med. Assoc.*, vol. 289, no. 19, pp. 2560–2572, May 2003.

[19] R. Barbieri, E. C. Matten, A. A. Alabi, and E. N. Brown, "A point-process model of human heartbeat intervals: new definitions of heart rate and heart rate variability," *Am. J. Physiol. Heart Circ. Physiol.*, vol. 288, no. 1, pp. H424–435, Jan. 2005.

[20] R. Barbieri and E. N. Brown, "Analysis of heartbeat dynamics by point process adaptive filtering," *IEEE Trans. Biomed. Eng.*, vol. 53, no. 1, pp. 4–12, Jan. 2006.

[21] Z. Zhou, J. Champagnat, and C. S. Poon, "Phasic and long-term depression in brainstem nucleus tractus solitarius neurons: differing roles of AMPA receptor desensitization," *J. Neurosci. Off. J. Soc. Neurosci.*, vol. 17, no. 14, pp. 5349–5356, Jul. 1997.

[22] V. Rauschel, A. Straube, F. Süß, R. Ruscheweyh, "Responsiveness of the autonomic nervous system during paced breathing and mental stress in migraine patients," *The Journal of Headache and Pain*. Vol. 16, no. 82, 2015.