Proceedings of the 2025 SICE Festival with Annual Conference
September 9-12, 2025, Chiang Mai, Thailand

Non-Invasive Alternatives to Microneurography: Evaluating Peripheral Arterial Stiffness and High-Frequency ECG-Derived Electro-Sympathetic Nerve Activity

Zu Soh[1†], Ryohei Kohama[1], Yoshifumi Nishio[2],Yoshiyuki Okada[2] and Toshio Tsuji[1]

[1]Graduate School of Advanced Science and Engineering, Hiroshima University, Hiroshima, Japan
[2]Department of Special Care Dentistry, Hiroshima University, Hiroshima, Japan
(Tel: +81-82-424-5763; E-mail: sozu@hiroshima-u.ac.jp (Z.S.), tsuji-c@bsys.hiroshima-u.ac.jp (T.T.))

Abstract: Microneurography remains the only method for measuring peripheral sympathetic nerve activity, but the percutaneous insertion of microneedles imposes a significant burden on patients. Therefore, a non-invasive alternative is needed. This study investigates the feasibility of using fingertip peripheral arterial stiffness and knee-lead electro-sympathetic nerve activity (ESNA) as non-invasive indices of peripheral sympathetic nerve activity. Seventeen healthy male participants underwent a cold pressor test (CPT) while ESNA (chest and knee leads) and fingertip peripheral arterial stiffness were recorded simultaneously. Additionally, in a separate invasive experiment with one male participant, muscle sympathetic nerve activity (MSNA) was recorded using microneurography, alongside the aforementioned non-invasive measures, to validate their potential as proxies for MSNA. The results showed significant increases in peripheral arterial stiffness ($p < 0.01$), chest-lead ESNA ($p < 0.01$), and knee-lead ESNA ($p < 0.01$) during cold water stimulation. Furthermore, in the invasive experiment, knee-lead ESNA exhibited a downward trend, similar to fibular MSNA, during cold water stimulation. These findings suggest that knee-lead ESNA and fingertip arterial stiffness reflect peripheral sympathetic responses and may serve as non-invasive alternatives to microneurography. Further studies with larger sample sizes are needed to validate their clinical utility.

Keywords: Sympathetic Nerve Activity, Arterial Stiffness, Electrocardiogram, Microneurography, Non-invasive Measure

1. INTRODUCTION

Microneurography remains the only technique capable of directly measuring muscle sympathetic nerve activity (MSNA) and skin sympathetic nerve activity (SSNA). It has been used to predict and assess the severity of conditions such as hypertension, obesity, cardiovascular diseases, and peripheral nerve disorders [1–4]. However, its invasiveness and the insertion of fine electrodes may cause pain or sensory abnormalities [5]. As a result, there is growing interest in developing non-invasive methods to assess sympathetic nerve activity. One such approach involves analyzing high-frequency components (500–1,000 Hz) of electrocardiographic signals (ECG) recorded from the skin, which have been reported to reflect cardiac sympathetic nerve activity (CSNA). While previous studies referred to this measure as skin sympathetic nerve activity (SKNA) [6], to avoid confusion with SSNA measured via microneurography, this paper instead terms it electro-sympathetic nerve activity (ESNA). However, the application of ESNA to peripheral sites beyond the heart remains unexplored.

This study focuses on peripheral sympathetic nerves, which regulate vascular tone and thermoregulation. The sympathetic nervous system transmits signals to the heart, blood vessels, and skin via efferent nerve fibers. CSNA increases heart rate [7], while MSNA induces vasoconstriction and inhibits digestion [8]. SSNA controls cutaneous blood flow and sweating [8].

Our research group previously proposed a log-linearized peripheral arterial viscoelastic model [9], which evaluates arterial properties using blood pressure and photoplethysmographic waveforms. Since sympathetic nerves regulate vascular tone, peripheral arterial stiffness is influenced by MSNA and SSNA. Studies have shown that peripheral arterial stiffness increases in response to sympathetic activation triggered by pain, anxiety [10, 11], unpleasant odors [12], electrical stimulation, and cold exposure [13]. Additionally, it correlates with the low-frequency components of MSNA [14]. However, the simultaneous measurement of MSNA and peripheral arterial stiffness has not been conducted, leaving their relationship unclear.

This study aims to evaluate non-invasive methods for assessing MSNA. To this end, we conducted a cold pressor test (CPT) [15–17] while simultaneously measuring peripheral arterial stiffness, ECG, and ESNA near the left knee. We first examined the responsiveness of these indices to cold stimulation. Then, MSNA was recorded via microneurography to analyze its relationship with knee-lead ESNA, assessing the feasibility of non-invasive MSNA measurement.

2. MATERIALS AND METHODS

2.1. Participants

A total of 17 healthy adult males (22.8 $\pm$ 0.71 years) participated in the ESNA measurement experiment, which aimed to simultaneously record ESNA and fingertip peripheral arterial stiffness. Additionally, one healthy adult male (41 years old) participated in the MSNA measurement experiment, where MSNA and

† Zu Soh is the presenter of this paper.

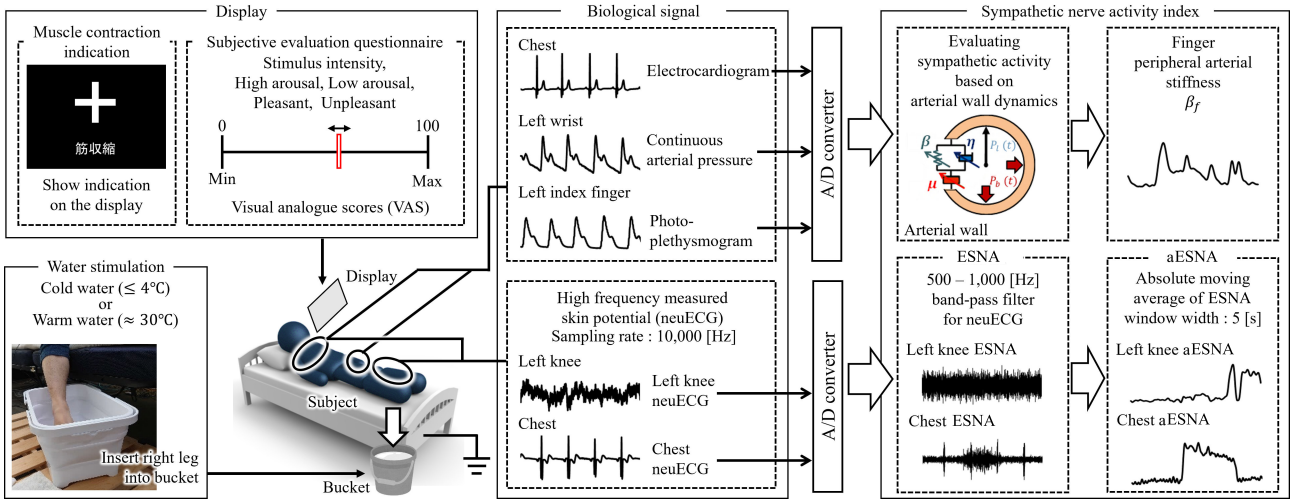

Fig. 1 Overview of the measurement system and physiological signal processing

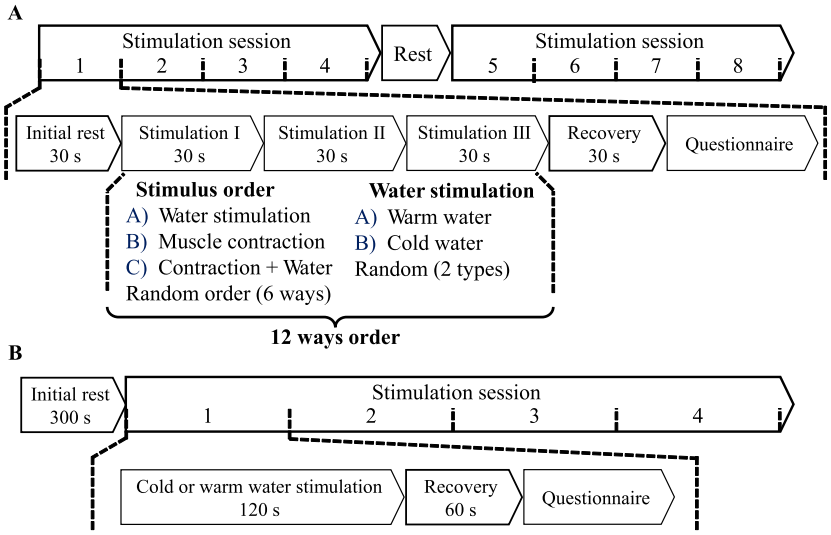

Fig. 2 Experimental protocols

ESNA were concurrently recorded using microneurography and surface electrodes, respectively.

All experimental procedures were approved by the Ethics Committee for Epidemiological Research at Hiroshima University (Approval No.: E-17-3). In accordance with the Declaration of Helsinki, informed consent was obtained from all participants prior to their involvement in the study.

2.2. Experimental Setup

The experimental system, illustrated in Fig. 1, consists of a stimulation unit, a physiological signal measurement unit, and a display for visual control and muscle contraction instructions.

In the stimulation presentation unit, two buckets containing cold and warm water were placed beside the bed. To administer thermal stimuli while minimizing participant movement, the experimenter immersed the participant ' s right leg into the designated bucket.

In the physiological signal measurement unit, continuous non-invasive blood pressure $P_b(t)$ from the left radial artery and three-lead electrocardiographic potentials from the chest were recorded using a physiological signal monitor (BP-608 Evolution II CS, Omron Colin, Japan). Additionally, photoplethysmographic signals $P_l(t)$ from the left index finger were measured using a pulse oximeter (OLV-4202, Nihon Kohden, Japan). These signals were digitized at a sampling rate of 1 kHz via an A/D converter (DC-300H, Nihon Kohden, Japan).

To measure ESNA, disposable electrodes (Bs-150, Nihon Kohden, Japan) were placed near the left fibular nerve at the knee and on the chest. The signals were processed using a biopotential coupler (PB-100, Nihon Kohden, Japan), a bioelectrical amplifier (AB-100, Nihon Kohden, Japan), and an A/D converter (DC-300H, Nihon Kohden, Japan) at a sampling rate of 10 kHz. To eliminate power line noise, all measurement devices, the recording PC, and the bed frame were commonly grounded.

The display presents either a white cross for visual fixation or muscle contraction instructions, depending on the experimental protocol.

2.3. Experimental Protocols

The protocols for the ESNA measurement experiment is shown in Fig. 2A. Each participant underwent eight stimulation sessions. In each session, after an initial 30-second resting period, three types of stimuli were presented for 30 seconds each: water stimulation, muscle contraction, and water stimulation during muscle contraction, followed by a 30-second recovery period. For water stimulation, cold water stimulation ($\leq$4°C) was used, along with warm water stimulation (30°C) as a comparison. Muscle contraction was applied to the left quadriceps femoris and right pectoralis major muscles.

The protocol for invasive MSNA recording via microneurography is shown in Fig. 2B. In this experiment, after attaching physiological measurement devices, a microelectrode was inserted into the fibular nerve. Each session began with a 300-second resting period, followed by 120 seconds of water stimulation and a 60-second recovery period, repeated four times.

All experiments were conducted in a temperature-controlled environment (approximately 25°C) maintained by air conditioning.

2.4. Analysis

The mechanical properties of peripheral arteries are described by the following log-linearized peripheral arterial viscoelastic model [9, 14].

$$P_b(t) = \mu \ddot{P}_l(t) + \eta \dot{P}_l(t) + \exp\left\{\beta P_l(t) + P_{b\beta_0} + P_{b\beta_{nl}}(P_l(t))\right\} \quad (1)$$

Here, μ, η, β represent the inertia, viscosity, and stiffness of the arterial wall, respectively. $P_b(t)$, $P_l(t)$, $\dot{P}_l(t)$, $\ddot{P}_l(t)$ denote the non-invasive continuous blood pressure, photoplethysmographic pulse wave, pulse wave velocity, and pulse wave acceleration at time t, respectively. $P_{b\beta_0}$ represents the mean circulatory filling pressure, while $P_{b\beta_{nl}}(P_l(t))$ denotes the distal arterial pressure. The measured $P_b(t)$ and $P_l(t)$ were substituted into Eq. (1) to estimate μ, η, β using the least squares method.

To improve estimation accuracy, peripheral arterial stiffness values with low reliability were excluded, and missing values were interpolated using a third-order spline method. Additionally, a low-pass filter with a cut-off frequency of 0.15 Hz was applied to eliminate respiratory influences.

To extract ESNA components, the recorded signals were filtered using a 500–1,000 Hz bandpass filter. Then, a 5-second moving absolute mean was computed, hereafter referred to as aESNA. The same processing was applied to invasively measured MSNA via microneurography, referred to as aMSNA. All signal processing was performed using LabChart 8.1.9 (ADInstruments, Dunedin, New Zealand).

To evaluate the response of each sympathetic nerve activity measure to CPT, the time-series signals of peripheral arterial stiffness and aESNA were divided into six stimulation intervals. Each 30-second stimulus presentation interval was adjusted to exclude the first and last

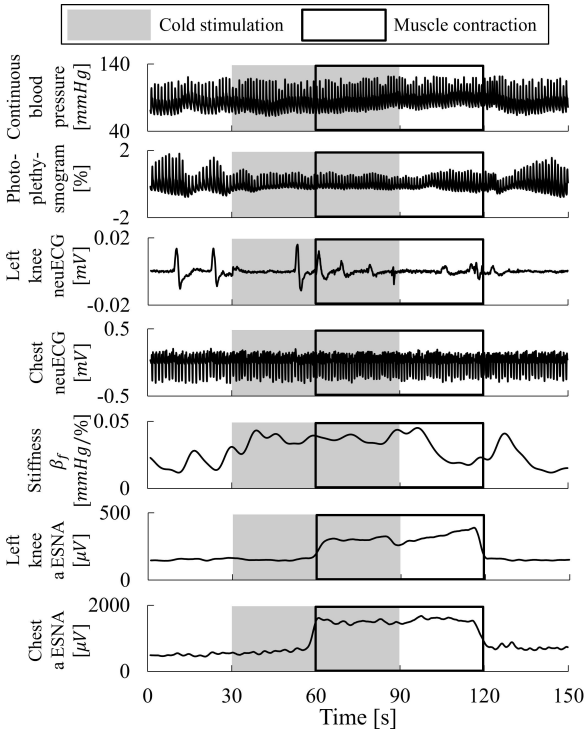

Fig. 3 Measured physiological signals and sympathetic activity indices of the ESNA measurement experiment

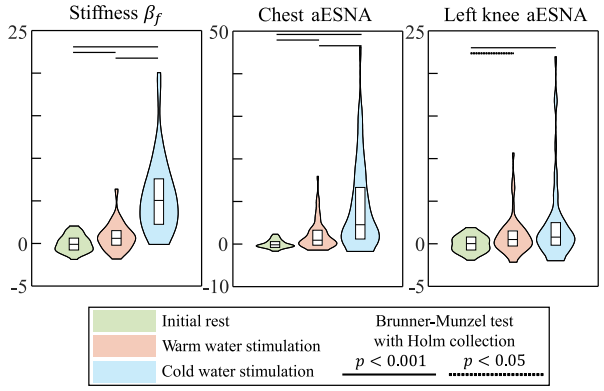

Fig. 4 Comparison of sympathetic nerve activity measures: Rest vs. warm water and rest vs. cold water stimulation

5 seconds to account for transient responses, resulting in a 20-second analysis window. Next, for each sympathetic nerve activity measure, the mean and variance of the resting period were calculated for each participant and used to normalize all measured values. The Brunner-Munzel test was then conducted to compare the standardized mean values between the resting period and each stimulation interval. Statistical significance was determined at $p < 0.05$ with Holm's correction for multiple comparisons. MATLAB2022a and R 4.2.2 were used for statistical analysis.

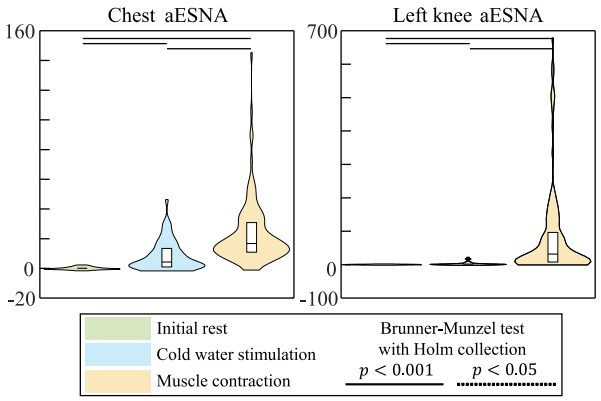

Fig. 5 Comparison of sympathetic nerve activity indices: rest vs muscle contraction period

3. RESULTS AND DISCUSSION

Figure 3 presents an example of physiological signals and sympathetic nerve activity indices recorded during the ESNA measurement experiment. The figure shows that during the cold water stimulation period, the amplitude of the fingertip photoplethysmographic waveform decreased, while peripheral arterial stiffness increased. Additionally, aESNA exhibited a slight increase during the cold water stimulation period and a substantial rise during the muscle contraction period. In contrast, continuous blood pressure remained largely unchanged.

Figure 4 presents the mean values of sympathetic nerve activity indices recorded during the resting period, warm water stimulation, and cold water stimulation. Sessions were deemed invalid and excluded from analysis if any mean value exceeded the first quartile minus 5 $\times$ the interquartile range or the third quartile plus 5 $\times$ the interquartile range. Based on this criterion, five warm water stimulation sessions and seven cold water stimulation sessions were excluded, resulting in 124 valid sessions out of 136 (17 participants $\times$ 4 sessions). The analysis revealed that both warm and cold water stimulation significantly increased all three sympathetic activity indices —peripheral arterial stiffness, chest-lead aESNA, and left knee-lead aESNA—compared to the resting period. The increase observed during cold water stimulation is consistent with a previous study reporting a cold water-induced rise in chest-lead aESNA, which reflects CSNA activation [6]. The elevation of chest-lead aESNA during warm water stimulation suggests that it may also induce sympathetic nerve activation. Furthermore, the increases in peripheral arterial stiffness and left knee-lead aESNA provide evidence that peripheral sympathetic nerves also responded to the stimulation, supporting the potential for non-invasive assessment of sympathetic function.

Figure 5 presents violin plots comparing the mean values of sympathetic nerve activity indices between the resting period and the muscle contraction period. Significant differences were observed in both chest-lead and left knee-lead aESNA between these periods. Given that muscle contractions generate electromyographic signals in a similar frequency range, this result suggests

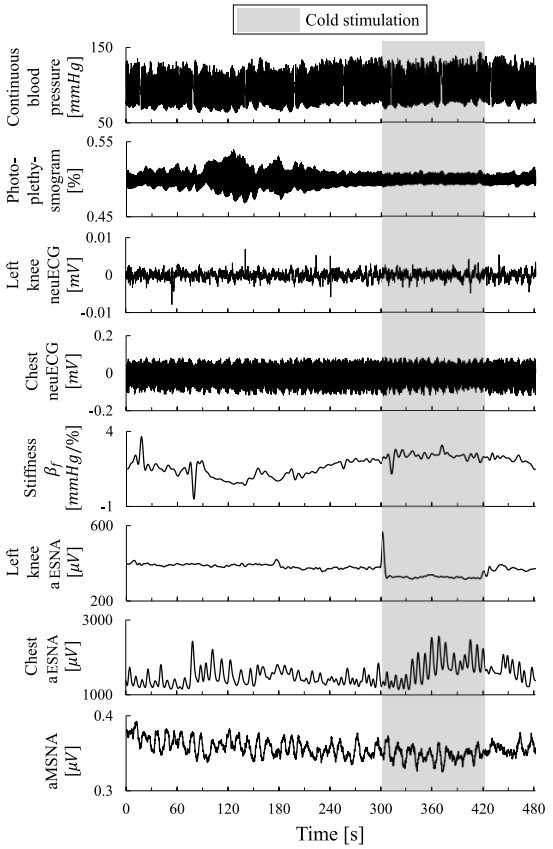

Fig. 6 Physiological signals and sympathetic nerve activity indices recorded during the invasive MSNA recording experiment

that electromyogram contamination may have influenced ESNA measurements. This highlights the importance of minimizing muscle contractions when performing non-invasive sympathetic nerve activity assessments.

Figure 6 presents the measurement results from the cold water stimulation session in the invasive MSNA recording experiment. The figure shows that during cold water stimulation, the amplitude of the fingertip photoplethysmographic waveform decreased, while peripheral arterial stiffness increased. A comparison of mean sympathetic nerve activity indices between the resting period and the cold water stimulation period revealed that upper-limb sympathetic nerve activity indices, including peripheral arterial stiffness and chest-lead aESNA, increased. In contrast, lower-limb sympathetic nerve activity indices, including left knee-lead aESNA and aMSNA, decreased. This consistent response pattern suggests that ESNA measurements at the left knee may reflect fibular nerve MSNA, supporting its potential for non-invasive MSNA estimation. By integrating findings from simultaneous measurements of ESNA and peripheral arterial stiffness, this study identifies two alternative, non-invasive indices for assessing peripheral sympathetic nerve activity: knee-lead aESNA and peripheral arterial stiffness. However, aESNA measurement requires careful experimental design and precise instructions to minimize contamination from electromyographic signals.

4. CONCLUSION

This study evaluated the feasibility of peripheral arterial stiffness and ESNA as non-invasive markers of peripheral sympathetic nerve activity using CPT experiments with simultaneous physiological recordings. Cold water stimulation increased both indices, suggesting their potential for assessment. ESNA was also affected by body movements and muscle contractions, which should be primarily controlled through procedural measures, such as clear instructions and pre-test practice sessions. A larger sample size may help reduce the statistical impact of such artifacts and also mitigate potential biases caused by the imbalance in participant numbers between the invasive and non-invasive groups. In addition, the applicability of the proposed method to females, children, and the elderly cannot be accurately assessed at this stage. Future studies will therefore include a more diverse population to validate the method across different demographic groups.

REFERENCES

[1] G. Grassi, A. Pisano, D. Bolignano, G. Seravalle, G. F. Quarti-Trevano, F. Mallamaci, C. Zoccali, and G. Mancia, " Sympathetic nerve traffic activation in essential hypertension and its correlates: systematic reviews and meta-analyses," *Hypertension*, vol. 72, no. 2, pp. 483–491, 2018.

[2] S. C. Malpas, " Sympathetic nervous system overactivity and its role in the development of cardiovascular disease, " *Physiol. Rev.*, vol. 90, no. 2, pp. 513–557, 2010.

[3] A. C. P. Barretto, A. C. Santos, R. Munhoz, M. U. P. B. Rondon, F. G. Franco, I. C. Trombetta, F. Roveda, L. N. J. de Matos, A. M. W. Braga, H. R. Middlekauff et al., " Increased muscle sympathetic nerve activity predicts mortality in heart failure patients, " *Int. J. Cardiol.*, vol. 135, no. 3, pp. 302–307, 2009.

[4] G. Grassi, M. Colombo, G. Seravalle, D. Spaziani, and G. Mancia, " Dissociation between muscle and skin sympathetic nerve activity in essential hypertension, obesity, and congestive heart failure, " *Hypertension*, vol. 31, no. 1, pp. 64–67, 1998.

[5] V. L. Meah, S. A. Busch, K. E. Jones, M. H. Davenport, and C. D. Steinback, " A review of acute responses, after-effects and chronic complications related to microneurography," *Clin. Neurophysiol.*, vol. 130, no. 10, pp. 1781–1788, 2019.

[6] T. Kusayama, J. Wong, X. Liu, W. He, A. Doytchinova, E. A. Robinson, D. E. Adams, L. S. Chen, S.-F. Lin, K. Davoren et al., " Simultaneous noninvasive recording of electrocardiogram and skin sympathetic nerve activity (neuECG), " *Nat. Protoc.*, vol. 15, no. 5, pp. 1853–1877, 2020.

[7] U. R. Acharya, K. P. Joseph, N. Kannathal, C. M. Lim, and J. S. Suri, " Heart rate variability: a review, " *Med. Biol. Eng. Comput.*, vol. 44, pp. 1031–1051, 2006.

[8] J. L. Greaney and W. L. Kenney, " Measuring and quantifying skin sympathetic nervous system activity in humans, " *J. Neurophysiol.*, vol. 118, no. 4, pp. 2181–2193, 2017.

[9] H. Hirano, T. Horiuchi, H. Hirano, Y. Kurita, T. Ukawa, R. Nakamura, N. Saeki, M. Yoshizumi, M. Kawamoto, and T. Tsuji, " A log-linearized peripheral arterial viscoelastic index and its application to endoscopic thoracic sympathectomy, " *Trans. Soc. Instrum. Control Eng.*, vol. 48, no. 11, pp. 731–739, 2012.

[10] H. Matsubara, H. Hirano, H. Hirano, Z. Soh, R. Nakamura, N. Saeki, M. Kawamoto, M. Yoshizumi, A. Yoshino, T. Sasaoka et al., " Quantitative evaluation of pain during electrocutaneous stimulation using a log-linearized peripheral arterial viscoelastic model, " *Sci. Rep.*, vol. 8, no. 1, p. 3091, 2018.

[11] Z. Xu, T. Sakagawa, A. Furui, S. Jomyo, M. Morita, M. Ando, and T. Tsuji, " Beat-to-beat estimation of peripheral arterial stiffness from local PWV for quantitative evaluation of sympathetic nervous system activity," *IEEE Trans. Biomed. Eng.*, vol. 69, no. 9, pp. 2806–2816, 2022.

[12] M. Totsuka, Z. Soh, T. Sasaoka, S. Yamawaki, and T. Tsuji, " Towards objective olfactory evaluation based on peripheral arterial stiffness and heart rate variability indices, " in *Proc. Annu. Int. Conf. IEEE Eng. Med. Biol. Soc. (EMBC)*, pp. 5618–5623, 2018.

[13] Z. Xu, R. Anai, H. Hirano, Z. Soh, and T. Tsuji, " Noninvasive characterization of peripheral sympathetic activation across sensory stimuli using a peripheral arterial stiffness index," *Front. Physiol.*, vol. 14, p. 1294239, 2024.

[14] T. Muneyasu, H. Hirano, A. Furui, Z. Soh, R. Nakamura, N. Saeki, Y. Okada, M. Kawamoto, M. Yoshizumi, A. Yoshino et al., " Cardiorespiratory synchronization and systolic blood pressure correlation of peripheral arterial stiffness during endoscopic thoracic sympathectomy, " *Sci. Rep.*, vol. 11, no. 1, p. 5966, 2021.

[15] J. Fagius, S. Karhuvaara, and G. Sundlöf, "The cold pressor test: effects on sympathetic nerve activity in human muscle and skin nerve fascicles, " *Acta Physiol. Scand.*, vol. 137, no. 3, pp. 325–334, 1989.

[16] R. G. Victor, W. N. Leimbach Jr, D. R. Seals, B. G. Wallin, and A. L. Mark, " Effects of the cold pressor test on muscle sympathetic nerve activity in humans, " *Hypertension*, vol. 9, no. 5, pp. 429–436, 1987.

[17] Y. Okada, S. S. Jarvis, S. A. Best, J. G. Edwards, J. M. Hendrix, B. Adams-Huet, W. Vongpatanasin, B. D. Levine, and Q. Fu, " Sympathetic neural and hemodynamic responses during cold pressor test in elderly blacks and whites, " *Hypertension*, vol. 67, no. 5, pp. 951–958, 2016.

ACKNOWLEDGMENTS

This work was supported by JSPS KAKENHI Grant Number 22H00197.