Validation of normalized pulse volume in the outer ear as a simple measure of sympathetic activity using warm and cold pressor tests: Towards applications in ambulatory monitoring

Validation of normalized pulse volume (NPV) in the outer ear as a simple measure of sympathetic activity using warm and cold pressor tests: Towards applications in ambulatory monitoring

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Abstract. Normalized pulse volume (NPV) derived from the ear has the potential to be a practical index for monitoring daily life stress. However, ear NPV has not yet been validated. Therefore, we compared NPV derived from an index finger using transmission photoplethysmography as a reference with NPV derived from a middle finger and four sites of the ear using reflection photoplethysmography during baseline and while performing cold and warm water immersion in 10 young and 6 middle-aged subjects. The results showed that *ln*NPV during cold water immersion as compared with baseline values was significantly lower, only at the index finger, the middle finger and the bottom of the ear-canal. Furthermore, *ln*NPV reactivities (Δ*ln*NPV; the difference between baseline and test values) from an index finger was significantly related to Δ*ln*NPV from the middle finger and the bottom of the ear-canal (young: r = 0.90 and 0.62, middle-aged: r = 0.80 and 0.58, respectively). In conclusion, these findings show that reflection and transmission photoplethysmography are comparable

methods to derive NPV in accordance with our theoretical prediction. NPV derived from the bottom of the ear-canal is a valid approach, which could be useful for evaluating daily life stress.

Key words: normalized pulse volume (NPV), ear-canal, photoplethysmography, cold pressor test, stress monitoring

1. Introduction

There have been sustained efforts, over several decades, to develop and improve non-invasive methods to investigate and monitor cardiovascular phenomena in various circumstances and such methods are of importance for clinical diagnosis and for monitoring therapies, as well as for research (Palatini *et al.*, 2011). Along with the development of non-invasive techniques for the measurement of blood pressure and blood flow, the investigation of pulse pressure (PP), pulse wave velocity (PWV) and pulse volume (PV) have also attracted considerable interest (Hamilton *et al.*, 2007; Millasseau *et al.*, 2000; Millasseau *et al.*, 2006; Xin *et al.*, 2007). PP, PWV and PV are recognized as having potentially useful relationships with arterial blood pressure, stroke volume and vascular mechanical properties, especially arterial tone and stiffness. Furthermore, peripheral PV has been found to provide information on autonomic vascular control (Miller and Ditto, 1989, 1991; Nitzan *et al.*, 1994; Javed *et al.*, 2010; Piepoli *et al.*, 1995).

Pulse volume can be measured quantitatively or detected qualitatively with a number of techniques, including air or water plethysmography, strain gauge plethysmography, electrical impedance plethysmography (EIP), and photoelectric plethysmography (PPG). Air and water plethysmography can provide relatively direct quantitative measurements of pulse volume and, by performing venous occlusion, they also allow estimation of blood flow, for example in a limb or digit. Strain gauge plethysmography and EIP have also been shown to allow both PV and blood flow estimations. PPG has been particularly popular for the assessment of peripheral perfusion due to its simplicity and convenience, in its simplest form only requiring the attachment of a light emitting diode (LED) and a photodetector (PD) (Challoner, 1979; Lindberg *et al.*, 1991). Although PPG has been shown to be capable of providing accurate quantitative data for heart rate and/or oxygen saturation, even under challenging conditions (Townshend *et al.*, 2006; Lopez-Silva *et al.*, 2012), the amplitude of the PPG signal hitherto has only provided predominantly qualitative data. There has therefore been significant interest in methods for normalizing the PPG amplitude and this interest continues.

The efforts to develop PPG have centred on the study of the component parts of the signals obtained by this technique, particularly their relationships with physical and physiological factors. Signals produced by PPG relate to the intensity of light, $\boldsymbol{I}$, that is detected following absorption and

scattering processes as the incident light, $\boldsymbol{I}_o$, propagates within tissues, and the Beer-Lambert-Bouguer law can be used to describe the relationship between $\boldsymbol{I}$ and $\boldsymbol{I}_o$. The PPG signal, which arises from $\boldsymbol{I}$, contains two basic components: firstly, a cardiac-synchronous pulse, the ac component, $\boldsymbol{I}_{ac}$, resembling the arterial blood pressure waveform, which is produced by the absorption of light by pulsating arterial and arteriolar blood; secondly, a slowly varying background signal, the dc component, $\boldsymbol{I}_{dc}$, that is related to absorption of light by tissues and venous blood. The PV signal derived from $\boldsymbol{I}$ in this way certainly has a relationship with the true arterial pulse volume in the region of tissue interrogated by the light emitter-detector pair, but the exact relationship varies according to a number of factors, including the emitter-detector spacing and configuration, the wavelength of light used, and the anatomical site used for the measurement. Thus direct comparisons of PV signals between different sites and different subjects are not possible. In order to address this problem, at least in part, the very earliest reports of the method included approaches for the normalization of the PV-related plethysmogram signals, that is the ac component, for example by expressing the volume pulse amplitude, $\Delta\boldsymbol{I}_{ac}$, as a percentage of the total photoelectric signal, $\boldsymbol{I}$, thus normalized pulse volume, NPV, was calculated as ($\Delta\boldsymbol{I}_{ac}/\boldsymbol{I}$) 100 % (Davis and Hertzman, 1957). The total photoelectric signal, $\boldsymbol{I}$, is often re-written as $\boldsymbol{I}_{dc}$ and this is a valid approximation since $\Delta\boldsymbol{I}_{ac}$ is very much smaller than $\boldsymbol{I}_{dc}$. Further approaches are to normalize the $\Delta\boldsymbol{I}_{ac}$ amplitude to the baseline $\Delta\boldsymbol{I}_{ac}$ amplitude, or to the dc component, $\boldsymbol{I}_{dc}$, (Sawada *et al.*, 2001; Tanaka and Sawada, 2003; Xin *et al.*, 2007) or, to enable waveform changes to be perceived more easily, to normalize the $\boldsymbol{I}_{ac}$ pulse width to the baseline pulse width value (Allen and Murray, 2003).

Traditionally, the photoplethysmogram has been obtained from the fingertip with the light source and detector in the transmission configuration. This site is particularly useful for studying autonomic control since the arteriolar vessels here are known to be very sensitive to vasoconstrictor stimuli (Sawada *et al.*, 2001). Other anatomical sites have also been used, in some cases, such as the toe and ear lobe, with transmission optodes and in other cases, including the forehead and oesophagus, using the reflectance configuration (Allen and Murray, 2000). Of all of these possible sites, the different locations on and in the ear can have several advantages. The vessels of the deep auricular artery, which originates from either the maxillary or the superficial temporal artery, ascend through the bony wall of the ear-canal from the parotid gland under the ear-canal, with its branches providing a supply to the lining and periphery of the ear-canal (Gray and Lewis, 1918; Wasicky and Pretterklieber, 2000). Thus, the photoplethysmogram can be obtained from there with the reflectance configuration. Furthermore there is relative freedom from motion artifacts produced, for example, during walking (Brodersen *et al.*, 2007; Vogel *et al.*, 2009; Wartzek *et al.*, 2009). Therefore, the collection of PPG-derived measurements from different regions of the ear could be well suited to the task of monitoring stress during normal daily life, especially through the use of the normalized pulse volume index, NPV.

Despite these potential advantages, the NPV derived from the ear has not yet been validated. Although Vogel and colleagues have validated the measurement of heart rate using the PV recorded in the ear-canal, they did not validate NPV (Vogel *et al.*, 2009; Wartzek *et al.*, 2009). Therefore, the purpose of our present study was to validate the ear NPV for evaluating stress. We used NPV derived from an index finger with the transmission mode PPG configuration (NPVt_IF) as a reference. We then compared the reference data with NPV values derived from a middle finger (NPVr_MF), from the top and bottom of the ear-canal (NPVr_ECT and NPVr_ECB, respectively), as well as from the upper and lower parts of the ear-auricle (NPVr_EAU and NPVr_EAL, respectively), in all cases using the reflection mode PPG configuration. We used cold pressor tests and warm water immersion as physiological challenges.

2. Methods

2.1. Participants

Two groups of participants, without known cardiovascular disorders, took part in this study. In the 'young' group there were 10 males, with a mean age of 21.9 ± 0.8 S.D. years, whilst the 'middle-aged' group contained 6 males, with a mean age of 48.8 ± 10.9 S.D. years. All subjects agreed to take part in this study voluntarily and signed an informed consent statement. The experimental design was approved by the ethics committee of the Faculty of Medicine of Kanazawa University.

2.2. Principle

In the present study we derived NPV data from the photoplethysmograph, which is based on the Beer-Lambert-Bouguer law that describes the relationship between the intensity of incident light ($\boldsymbol{I}_o$) and transmitted light ($\boldsymbol{I}$), when the light passes through a non-scattering medium. If it is assumed that this law can be applied for transmission measurements in the fingertip, the relationship between the pulsatile change in the arterial blood volume, $\Delta \boldsymbol{V}_a$, and the intensity of the transmitted light could be derived. Within this context, the use of the transmission PPG obtained from a finger to derive the NPV has been analyzed theoretically with the application of the Beer-Lambert-Bouguer law (Sawada *et al.*, 2001; Tanaka and Sawada, 2003). It was shown (Sawada *et al.*, 2001; Tanaka and Sawada, 2003) that NPV could be derived simply by normalizing the magnitude of the ac component to the mean dc component, giving the following relationship:

$$\mathrm{NPV} = \Delta \boldsymbol{I}_{ac} / \boldsymbol{I}_{dc} \, (\propto \Delta V_a) \tag{1}$$

Equation (1) shows that the peak-to-trough magnitude of the ac component ($\Delta \boldsymbol{I}_{ac}$) is essentially corrected by the mean intensity of transmitted light, which is the mean dc component ($\boldsymbol{I}_{dc}$) photoplethysmographically detected from the finger. Thus, NPV is in direct proportion to the pulsatile component of the arterial blood volume, ΔV_a.

However, the validity of equation (1) must be questioned since it is based on the assumption of a non-scattering medium; in fact, when light passes through biological tissues it is extensively scattered as well as absorbed. Thus, the modified Beer-Lambert-Bouguer law, which includes the influence of light-scattering, should be considered (Nishimura *et al.*, 1996; Twersky, 1962, 1970).

According to the modified Beer-Lambert-Bouguer law for light-scattering, the relation between $\boldsymbol{I}_o$ and $\boldsymbol{I}$, when the light passes through absorptive and scattering homogeneous media, can be expressed as:

$$-\ln\left(\boldsymbol{I}/\boldsymbol{I}_o\right) = \varepsilon CkV + S \tag{2}$$

where ε is the molar absorptivity of the medium, C is the molar concentration of the medium. k is a volume correction factor, which is defined as the average of the differential path-length of light caused by scattering in the medium between light source and photodetector. If the light passes through a non-scattering medium $k = 1$, whilst when there is scattering $k > 1$. V is the volume of the medium where there is non-scattering (i.e., $k = 1$), and S is the attenuation due to scattering. Equation 2 can be rewritten as:

$$-\ln\left(\boldsymbol{I}/\boldsymbol{I}_o\right) = \varepsilon CV_c + S \tag{3}$$

where V_c is the corrected volume: $V_c = kV$

We now consider the fingertip being composed of three components: arterial blood; venous blood; and ischemic tissue. If we assume that the modified Beer-Lambert-Bouguer law holds in the fingertip, the relation between $\boldsymbol{I}_o$ and $\boldsymbol{I}$ can be expressed by the following equation:

$$-\ln\left(\boldsymbol{I}/\boldsymbol{I}_o\right) = \varepsilon_a C_a V_{ca} + \varepsilon_v C_v V_{cv} + \varepsilon_t C_t V_{ct} + S_{a+v+t} \tag{4}$$

Here ε_a, ε_v, and ε_t, are the absorptivity of the arterial and venous blood, and combined tissue other than blood, respectively. Similarly, C_a, C_v, and C_t, are the hematocrit (i.e. concentration of hemoglobin) of the arterial and venous blood, as well as the concentration of the combined tissue absorbers, respectively. V_{ca}, V_{cv}, and V_{ct}, are the correction volume of the arterial blood, venous blood, and bloodless tissue, respectively. S_{a+v+t} is the attenuation due to scattering under a normal circulatory state in which arterial and venous blood and bloodless tissue all contribute to scatter.

Next, we consider the situation in which the finger is made to be ischemic, there then being only the bloodless tissue present. Now the relationship between $\boldsymbol{I}_o$ and the transmitted light intensity, $\boldsymbol{I}_t$, is given as:

$$-\ln\left(\boldsymbol{I}_t/\boldsymbol{I}_o\right) = \varepsilon_t C_t V_{ct} + S_t \tag{5}$$

where S_t is the attenuation due to scattering by the bloodless tissue under an ischemic circulatory state.

Subtracting equation (5) from equation (4), and using the exponential transform, yields the following equation:

$$\boldsymbol{I}/\boldsymbol{I}_t = e^{\left(-\varepsilon CV_c + S\right)} \tag{6}$$

where V_c is the corrected volume of the total blood, ε is the mean absorptivity of the total blood and S

is the scattering attenuation of the total blood: $V_c = V_{ca} + V_{cv}$, $\varepsilon = (\varepsilon_a V_{ca} + \varepsilon_v V_{cv}) / V_c$, and $S = S_{a+v+t} - S_t$, respectively. C_a and C_v are considered to be constant and equal to the mean concentration of the total blood (C) under a normal circulatory state, and these change only a few percent from rest to a mental stress state (Muldoon *et al.*, 1992), thus $C = C_a = C_v$.

Taking the first derivative of $\boldsymbol{I}/\boldsymbol{I}_t$ with respect to V_c in equation (6) leads to:

$$\mathrm{d}\left(\boldsymbol{I}/\boldsymbol{I}_t\right)/\mathrm{d}V_c = -\varepsilon C\left(\boldsymbol{I}/\boldsymbol{I}_t\right) \tag{7}$$

and this subsequently leads to:

$$\Delta V_c = -\left(\varepsilon C\right)^{-1} \Delta \boldsymbol{I}/\boldsymbol{I} \tag{8}$$

Here, ΔV_c consists of the volume correction factor and the pulsatile components of the total blood volume: $\Delta V_c = k\Delta V$. Although the total blood volume consists of the mean and pulsatile components of the arterial blood volume, as well as of the venous blood volume, pulsation is only observed in arterial and arteriolar blood, so ΔV and the corresponding $\Delta \boldsymbol{I}$ may be rewritten as ΔV_a and $\Delta \boldsymbol{I}_{ac}$, respectively. Correspondingly, $\boldsymbol{I}$ may be rewritten as $\boldsymbol{I}_{dc}$. Thus:

$$\Delta V_a = -\left(k\varepsilon C\right)^{-1} \Delta \boldsymbol{I}_{ac}/\boldsymbol{I}_{dc} \tag{9}$$

The normalized pulse volume, NPV, being equal to the ratio of the ac and dc PPG components, $\Delta \boldsymbol{I}_{ac}/\boldsymbol{I}_{dc}$, is therefore simply derived from equation (9). It can be seen that NPV is related to the pulsatile component of the arterial blood volume, ΔV_a. Furthermore, there may be considered to be direct proportionality on the assumption that the mean absorptivity (ε), the hematocrit (C) and the volume correction factor (k) of the total blood are constant. Conditions under which the validity of such an assumption can be assured will be relatively easily met by adopting short term mental stress testing (Muldoon *et al.*, 1992) or by using an isosbestic wavelength (e.g., 805nm). Thus, from equation (9), we may state the modified NPV (mNPV):

$$\mathrm{mNPV} = \Delta \boldsymbol{I}_{ac}/\boldsymbol{I}_{dc} \left(\propto \Delta V_a\right) \tag{10}$$

This theoretical derivation, which utilizes the modified Beer-Lambert-Bouguer law, serves to validate the use of the mNPV mathematical relationship in real biological tissues where there are both absorptive and scattering processes. This provides more confidence in the use of the NPV index when the method is applied to reflection PPG, where multiple scattering events are key aspects of photon propagation.

It is noteworthy, however, that, regardless of the starting point, the final result as seen in equation (10) is the same as equation (1). Therefore, we are justified in using the term “NPV” instead of “mNPV”. Nevertheless, although the derivation described above confirms that there is no need to distinguish between the NPV using the transmission mode PPG (NPVt) and that using the reflection mode PPG (NPVr), for completeness in the present paper we continue to specify NPVt and NPVr.

2.3. Measurements and instruments

Near-infrared LEDs (940nm, VSMB1940X01, Vishay, USA) were used as light sources and PIN photodiodes (BPW34FS, OSRAM, Germany) as the detectors. Figure 1 shows the attachment configuration of sensors for the measurement of NPV. An LED and a photodiode were placed on opposite sides of the distal part of the index finger of the left hand (IF), in the transmission mode PPG. For all other measurement sites the reflection mode PPG was used, with the LED and photodiode of each pair being placed side-by-side. The reflection sites were: at the distal part of the middle finger of the left hand (MF) (see figure 1 panel A); the top and bottom of the ear-canal (ECT and ECB, respectively); as well as the upper and lower part of the ear-auricle on the left ear (EAU and EAL, respectively) (see figure 1 panel B). The LEDs used in the ear were operated in the pulsatile mode, receiving 250us pulses at 16.6ms intervals, to allow simultaneous measurement without interference among the four channels in the ear. The ac and dc photodiode outputs were amplified with gains of 150 and 100 respectively using a high precision OP-Amp. The signals were recorded by a data acquisition card (BNC-2111, National Instruments, USA) with 1kHz sampling rate, for storage and off-line analysis using LabVIEW (National Instruments, Austin, USA). We carefully positioned the reflection type sensors in the ear-canal and the ear concha, and then secured them by infusing an impression material (detax addition, DETAX, Germany).

For calculating NPV, we measured PV and $\boldsymbol{I}_{dc}$ at the index finger using the transmission PPG (PVt_IF and $\boldsymbol{I}_{dc}$t_IF). Also, we measured PV and $\boldsymbol{I}_{dc}$ at the MF (PVr_MF and $\boldsymbol{I}_{dc}$r _MF), the ECT (PVr _ECT and $\boldsymbol{I}_{dc}$r_ECT), the ECB (PVr_ECB and $\boldsymbol{I}_{dc}$r_ECB), the EAU (PVr_EAU and $\boldsymbol{I}_{dc}$r_EAU), and the EAL (PVr_EAL and $\boldsymbol{I}_{dc}$r_EAL) using the reflection PPG.

2.4. Procedure

Participants were requested to refrain from drinking alcohol and carrying out vigorous exercise for at least 1-day before the experiment, and from smoking, eating, and taking caffeinated beverages for least 2-hours before. Participants were subjected to two challenges: cold (4°C) pressor test (CPT) and warm (37°C) water immersion (WWI) of the right hand up to the wrist to induce peripheral vasoconstriction (Allen *et al.*, 1992; Allen *et al.*, 1997). The experiments were performed in a quiet, air-conditioned laboratory (temperature: 26 °C, humidity: 53 %). At the start the participants sat on a chair to relax, and then the sensors were attached on the index and middle finger of the left hand, as well as the ear-canal and the auricle of the left ear. The stages of the experiment were carried out in the following order while the participants sat quietly: (a) adaptation for 10 min; (b) baseline (BS) for 5 min; (c) the CPT for 90 s; (d) rest for 5 min; (e) the WWI for 90 s.

2.5. Data analysis

All the stored data were equally subject to 30 Hz low pass digital filtering based upon the fast Fourier transform algorithm. Logarithmic transformation was applied to PV, $\boldsymbol{I}_{dc}$, and NPV to normalize the

distribution (*ln*PV, $ln\boldsymbol{I}_{dc}$, and *ln*NPV, respectively). *ln*PV, $ln\boldsymbol{I}_{dc}$, and *ln*NPV values were averaged over 10 s, and these values further averaged to produce BS, first (0-30 s), second (30-60 s), and third (60-90 s) parts of the CPT as well as WWI values, respectively. These values were separated into two series: the CPT series and the WWI series. Each series was compared statistically by means of the one-way analysis of variance (ANOVA), and then Ryan's method applied for the comparisons, respectively.

Furthermore, *ln*PV and *ln*NPV reactivities (Δ*ln*PV and Δ*ln*NPV) were calculated by subtracting the BS values from the CPT and WWI values. And then, Pearson's correlation analysis was performed between Δ*ln*NPVt_IF as a reference with Δ*ln*NPVr_MF, Δ*ln*NPVr_ECT, Δ*ln*NPVr_ECB, Δ*ln*NPVr_EAU, and Δ*ln*NPVr_EAL, respectively, as well as, between Δ*ln*NPVt_IF as a reference with Δ*ln*PVr_ECT, Δ*ln*PVr_ECB, Δ*ln*PVr_EAU, and Δ*ln*PVr_EAL, respectively.

3. Results

3.1. lnPV and $ln\boldsymbol{I}_{dc}$, as well as lnNPV in the young group

During the CPT, mean and S.D. values of *ln*PV and $ln\boldsymbol{I}_{dc}$, as well as *ln*NPV, are summarized in table 1 for the index finger, the middle finger, the top of the ear-canal, the bottom of the ear-canal, the upper part of the ear-auricle, and the lower part of the ear-auricle, respectively. In the same way, during the WWI, mean and S.D. values of *ln*PV and $ln\boldsymbol{I}_{dc}$, as well as *ln*NPV, are summarized in table 2.

3.1.1. Index finger. During the CPT, the one-way ANOVA revealed the main effect of period: $F(3,27) = 75.44$, $p < 0.001$ for *ln*PVt_IF; $F(3,27) = 79.48$, $p < 0.001$ for $ln\boldsymbol{I}_{dc}$t_IF; $F(3,27) = 107.80$, $p < 0.001$ for *ln*NPVt_IF. Furthermore, significant differences among each period were found by Ryan's method: BS > CPT1, CPT2, and CPT3 for *ln*PVt_IF and *ln*NPVt_IF; BS < CPT1, CPT2, and CPT3 for $ln\boldsymbol{I}_{dc}$t_IF. Additionally, during the WWI, ANOVA revealed the main effect of period: $F(3,27) = 11.46$, $p < 0.001$ for *ln*PVt_IF; $F(3,27) = 16.51$, $p < 0.001$ for $ln\boldsymbol{I}_{dc}$t_IF; $F(3,27) = 16.57$, $p < 0.001$ for *ln*NPVt_IF. Significant differences among each period were found by Ryan's method: BS > WWI1 for *ln*PVt_IF and *ln*NPVt_IF; BS < WWI1, WWI2, and WWI3 for $ln\boldsymbol{I}_{dc}$t_IF.

3.1.2. Middle finger. During the CPT, the one-way ANOVA revealed the main effect of period: $F(3,27) = 80.77$, $p < 0.001$ for *ln*PVr_MF; $F(3,27) = 17.74$, $p < 0.001$ for $ln\boldsymbol{I}_{dc}$r_MF; $F(3,27) = 80.68$, $p < 0.001$ for *ln*NPVr_MF. Furthermore, significant differences among each period were found by Ryan's method: BS > CPT1, CPT2, and CPT3 for *ln*PVr_MF and *ln*NPVr_MF; BS < CPT1, CPT2, and CPT3 for $ln\boldsymbol{I}_{dc}$r_MF. Also, during the WWI, ANOVA revealed the main effect of period: $F(3,27) = 18.58$, $p < 0.001$ for *ln*PVr_MF; $F(3,27) = 3.53$, $p < 0.05$ for $ln\boldsymbol{I}_{dc}$r_MF; $F(3,27) = 18.52$, $p < 0.001$ for *ln*NPVr_MF. Significant differences among each period were found by Ryan's method: BS > WWI1 for the *ln*PVr_MF and *ln*NPVr_MF.

3.1.3. The top of the ear-canal. During the CPT, the one-way ANOVA revealed the main effect of period: F (3,27) = 4.10, $p < 0.05$ for *ln*PVr_ECT; F (3,27) = 4.33, $p < 0.05$ for $ln\boldsymbol{I}_{dc}$r_ECT; F (3,27) = 4.33, $p < 0.1$ for *ln*NPVr_ECT. Significant differences among each period were found by Ryan's method: BS > CPT1 and CPT2 for *ln*PVr_ECT; BS < CPT1, CPT2 and CPT3 for $ln\boldsymbol{I}_{dc}$r_ECT. Furthermore, during WWI, ANOVA revealed the main effect of period: F (3,27) = 5.13, $p < 0.05$ for $ln\boldsymbol{I}_{dc}$r_ECT. Significant differences among each period were found by Ryan's method: BS < WWI1, WWI2, and WWI3 for $ln\boldsymbol{I}_{dc}$r_ECT.

3.1.4. The bottom of the ear-canal. During the CPT, the one-way ANOVA revealed the main effect of period: F (3,27) = 8.43, $p < 0.001$ for *ln*PVr_ECB; F (3,27) = 8.44, $p < 0.001$ for *ln*NPVr_ECB. Significant differences among each period were found by Ryan's method: BS > CPT1, CPT2 and CPT3 for *ln*PVr_ECB and *ln*NPVr_ECB.

3.1.5. The upper part of the ear-auricle. During the CPT, the one-way ANOVA revealed the main effect of period: F (3,27) = 3.05, $p < 0.05$ for *ln*PVr_EAU; F (3,27) = 2.79, $p < 0.1$ for $ln\boldsymbol{I}_{dc}$r_EAU; F (3,27) = 2.55, $p < 0.1$ for *ln*NPVr_EAU.

3.1.6. The lower part of the ear-auricle. During the CPT, the one-way ANOVA revealed the main effect of period: F (3,27) = 3.47, $p < 0.05$ for *ln*PVr_EAL; F (3,27) = 3.61, $p < 0.05$ for *ln*NPVr_EAL. Significant differences among each period were found by Ryan's method: BS > CPT2 for *ln*PVr_EAL and *ln*NPVr_EAL.

3.2. lnPV and $ln\boldsymbol{I}_{dc}$, as well as lnNPV in the middle-aged group

During the CPT, mean and S.D. values of *ln*PV and $ln\boldsymbol{I}_{dc}$, as well as *ln*NPV, are summarized in table 3 for the index finger, the middle finger, the top of the ear-canal, the bottom of the ear-canal, the upper part of the ear-auricle, and the lower part of the ear-auricle, respectively. In the same way, during the WWI, mean and S.D. values of *ln*PV and $ln\boldsymbol{I}_{dc}$, as well as *ln*NPV, are summarized in table 4.

3.2.1. Index finger. During the CPT, the one-way ANOVA revealed the main effect of period: F (3,15) = 32.95, $p < 0.001$ for *ln*PVt_IF; F (3,15) = 76.24, $p < 0.001$ for $ln\boldsymbol{I}_{dc}$t_IF; F (3,15) = 50.85, $p < 0.001$ for *ln*NPVt_IF. Furthermore, significant differences among each period were found by Ryan's method: BS > CPT1, CPT2, and CPT3 for *ln*PVt_IF and *ln*NPVt_IF; BS < CPT1, CPT2, and CPT3 for $ln\boldsymbol{I}_{dc}$t_IF. Additionally, during the WWI, ANOVA revealed the main effect of period: F (3,15) = 4.40, $p < 0.05$ for *ln*PVt_IF; F (3,15) = 4.61, $p < 0.05$ for $ln\boldsymbol{I}_{dc}$t_IF; F (3,15) = 4.63, $p < 0.05$ for *ln*NPVt_IF. Significant differences among each period were found by Ryan's method: BS > WWI1 for *ln*PVt_IF and *ln*NPVt_IF; BS < WWI1 for $ln\boldsymbol{I}_{dc}$t_IF.

3.2.2. Middle finger. During the CPT, the one-way ANOVA revealed the main effect of period: F (3,15) = 45.65, $p < 0.001$ for *ln*PVr_MF; F (3,15) = 30.32, $p < 0.001$ for $ln\boldsymbol{I}_{dc}$r_MF; F (3,15) = 49.38, $p < 0.001$ for *ln*NPVr_MF. Furthermore, significant differences among each period were found by

Ryan's method: BS > CPT1, CPT2, and CPT3 for *ln*PVr_MF and *ln*NPVr_MF; BS < CPT1, CPT2, and CPT3 for $ln\boldsymbol{I}_{dc}$r_MF. Also, during the WWI, ANOVA revealed the main effect of period: F (3,15) = 6.45, $p < 0.01$ for *ln*PVr_MF; F (3,15) = 4.84, $p < 0.05$ for $ln\boldsymbol{I}_{dc}$r_MF; F (3,15) = 6.49, $p < 0.01$ for *ln*NPVr_MF. Significant differences among each period were found by Ryan's method: BS > WWI1 for the *ln*PVr_MF and *ln*NPVr_MF; BS < WWI1 for $ln\boldsymbol{I}_{dc}$r_MF.

3.2.3. The bottom of the ear-canal. During the CPT, the one-way ANOVA revealed the main effect of period: F (3,15) = 4.38, $p < 0.05$ for *ln*PVr_ECB; F (3,15) = 4.15, $p < 0.05$ for *ln*NPVr_ECB. Significant differences among each period were found by Ryan's method: BS > CPT2 for *ln*PVr_ECB and *ln*NPVr_ECB.

3.3. Correlation between the reactivities of lnNPV from an index finger as a reference with the reactivities of lnPVs and lnNPVs at the ear

During the CPT and WWI, Pearson's correlation coefficient between Δ*ln*NPV at the index finger as a reference with Δ*ln*PV and Δ*ln*NPV from the middle finger and the different placements of the ear are presented in the young and middle-aged groups, respectively (table 5). In both age groups, the significant relationships are observed between Δ*ln*NPVt_IF with Δ*ln*NPVr_MF, during the CPT and WWI. Furthermore, in the young group, the significant relationships are observed between Δ*ln*NPVt_IF with Δ*ln*PVr_ECB, Δ*ln*NPVr_ECT, and Δ*ln*NPVr_ECB, during the CPT. In the middle-aged group, the significant relationships are observed between Δ*ln*NPVt_IF with Δ*ln*PVr_ECB, Δ*ln*PVr_EAL, Δ*ln*NPVr_ECB, and Δ*ln*NPVr_EAL, during the CPT.

Figure 2 shows the two scatter plots for the correlation and the linear regression in the young and middle-aged groups during the CPT between Δ*ln*NPVt_IF (x-axis) with Δ*ln*NPVr_MF (y-axis) (see figure 2 panel A), and Δ*ln*NPVr_ECB (y-axis) (see figure 2 panel B).

4. Discussion

Peripheral pulse volume has previously been used to provide information on autonomic vascular control (Miller and Ditto, 1989, 1991) and the PPG has long been recognized as offering a convenient tool for the assessment of peripheral perfusion (Challoner, 1979). However, normalized pulse volume (NPV) has been reported as being a more valid measure for the assessment of α-adrenergically mediated peripheral vascular tone (Sawada *et al.*, 2001). Whilst the finger has traditionally been used as the most appropriate anatomical site for measuring the PPG, other sites are clearly possible (Allen and Murray, 2003), and the ear could be advantageous in certain situations. The vessels of the deep auricular artery pass between the tragus and eardrum, with its branches providing a supply to the skin of the cartilaginous ear-canal (Gray and Lewis, 1918). As compared with measurements in the finger or the limbs PPG recorded from the ear can be relatively free from motion artifacts (Vogel *et al.*, 2009). Thus, the collection of PPG-derived measurements from the ear could be well suited to the task

of monitoring stress during normal daily life, for example through the use of the NPV index (Sawada *et al.*, 2001). However, the NPV derived from the ear has not yet been validated and this was the purpose of the work described herein. In order to attempt to validate the NPV derived from PPG measurements at different sites in the ear, we have compared *ln*PV, $ln\boldsymbol{I}_{dc}$, and *ln*NPV with reference data derived from transmission PPG measurements in an index finger during cold pressor tests, CPT, and warm water immersion, WWI, as physiological challenges. Specifically we have compared the reference NPV values with the reflection PPG measurements, *ln*PVr and *ln*NPVr at different locations of the ear.

We have calculated NPV by dividing PV by the so-called dc component of the PPG. Naturally, if the dc component is essentially constant, NPV values will be directly proportional to the PV and the calculation of NPV is seemingly unnecessary. However, PV itself is affected by several instrumental and anatomical factors, including amplifier gain and skin color, and the normalization process to derive NPV minimizes the adverse influence of these factors (Sawada *et al.*, 2001). In the present study, we kept these variables constant, so the correlation coefficient of Δ*ln*NPVt_IF versus Δ*ln*NPVr_ECB was comparable to that of Δ*ln*NPVt_IF versus Δ*ln*PVr_ECB (table 5) during the cold pressor tests. However, not all measurements can be conducted under such controlled conditions, especially if the system is to be used during normal daily life. Therefore, NPV derived at the ear could be more suited to the task of monitoring stress than PV.

Our results show that, among the ear sites considered, the NPV values derived from reflection PPG measurements at the bottom of the ear-canal (NPVr_ECB) have the closest relationships with the reference data regardless of age group. This is judged, firstly, by the significant decreases that are observed in *ln*NPVr_ECB and *ln*PVr_ECB from the baseline values to the values obtained during the CPT in both age groups (table 1 and table 3). These decreases of *ln*NPVr_ECB and *ln*PVr_ECB indicate vasoconstriction by sympathetic activity during the exposure to the stressful stimuli (Sawada *et al.*, 2001; Tanaka and Sawada, 2003). Secondly, good relationships were observed during the CPT between Δ*ln*NPVt_IF as the reference with Δ*ln*NPVr_ECB, and Δ*ln*PVr_ECB in both age groups (table 5). These relationships could be explained by the reported location of the origin of the deep auricular artery on the lining of the ear-canal. The vessels of the deep auricular arteries ascend through the bony wall of the ear-canal from the parotid gland under the ear-canal, with branches providing a supply to the lining of the ear-canal (Gray and Lewis, 1918). That is, the change of the blood volume at the bottom of the ear-canal is larger than the change of the blood volume at the different locations in and around the ear. Taken together, these findings suggest that NPVr_ECB could be used as an alternative to NPVt_IF for evaluating physiological and psychological stress regardless of age.

The results for NPV values derived from measurements at the top of the ear-canal (NPVr_ECT), were somewhat inferior to those from the bottom of the ear-canal, NPVr_ECB. Although *ln*NPVr_ECT and *ln*PVr_ECT fell from baseline to CPT (table 1), the decreases were smaller than

those of *ln*NPVr_ECB and *ln*PVr_ECB. These findings indicate that the sensitivity of NPVr_ECT during CPT was lower than that of NPVr_ECB. Furthermore, during the CPT, the correlation of Δ*ln*NPVt_IF versus Δ*ln*NPVr_ECT was lower than Δ*ln*NPVt_IF versus Δ*ln*NPVr_ECB as demonstrated by both analyses (table 5). Therefore, although NPVr_ECT could also be used as an alternative to NPVt_IF, if NPVr_ECB is available then it is to be preferred.

In contrast to the normalized pulse volume data derived from the top and bottom of the ear-canal, NPVr_ECT and NPVr_ECB respectively, the indices derived from measurements at the upper (EAU) and lower (EAL) regions of the ear-auricle, gave poorer results. Although the cold pressor tests induced changes in the PV and NPV indices the magnitudes of these changes were much less pronounced than those seen with the ear-canal measurements. Falls in both PV and NPV indices measured at the lower ear-auricle were produced by the CPT, but these were much smaller than those obtained in the ear-canal (table 1). For upper ear-auricle measurements, during the cold pressor tests *ln*NPVr_EAU and *ln*PVr_EAU did not fall significantly from the baseline values (table 1). Furthermore, the cold pressor tests did not produce any statistically significant relationships between Δ*ln*NPVt_IF and either Δ*ln*NPVr_EAU, or Δ*ln*NPVr_EAL (table 5). These results suggest that the NPV and PV indices derived from PPG measurements at the upper and lower regions of the ear-auricle, NPVr_EAU and NPVr_EAL respectively, are not ideally suited to the task of monitoring stress. This could imply that the supply to the auricle regions is more sparse; there is perhaps an insufficient arterial supply to allow the measurement of NPV from those regions (Gray and Lewis, 1918).

It is of significance that the results show that the pattern of the autonomic vascular control in the ear appears to be more complex than that in the finger. In general, autonomic control processes in the peripheral vasculature appears to mitigate the changes induced by stimuli (Miller and Ditto, 1989, 1991; Xin *et al.*, 2007). For example, the PV and total blood volume in the finger decreased during an exposure to stressful stimuli (Sawada *et al.*, 2001; Tanaka and Sawada, 2003). In the present study, the decreases seen in the *ln*PV and $ln\boldsymbol{I}_{dc}$ indices derived from the finger during the CPT could be comparable to those findings. On the other hand, the *ln*PV in the ear-canal was found to fall during the CPT, while the $ln\boldsymbol{I}_{dc}$ in the ear-canal was maintained nearly constant during the CPT, although a significant change of $ln\boldsymbol{I}_{dc}$ at the top of the ear-canal was observed (table 1). The nearly constant $ln\boldsymbol{I}_{dc}$ observed in the ear could be explained by the notion that the blood flow in the head and neck circulation is maintained relatively constant by cerebral autoregulation (Panerai, 1998). So, the pattern of the autonomic vascular control in the ear-canal might be a mixture of the maintenance of total blood volume and vasoconstriction induced by exposure to stressful stimuli.

In addition to these main results, there are other points related to ear NPV to consider. Firstly, we have performed the CPT and WWI in the right hand up to the wrist to induce peripheral vasoconstriction. As a result, *ln*NPVt_IF and *ln*NPVr_MF, as well as *ln*PVr_IF and *ln*PVr_MF were

significantly decreased from BS to CPT (table 1 and table 3). In fact, the CPT has been frequently used to induce peripheral vasoconstriction (Allen *et al.*, 1992). Consistent with those findings, the same tendencies were observed from BS to WWI (table 2 and table 4), although the changes of *ln*NPVt_IF and *ln*NPVr_MF, as well as *ln*PVr_IF and *ln*PVr_MF were smaller than the change of those from BS to CPT. Therefore, these results suggest that the CPT and WWI successfully produced peripheral vasoconstriction.

Secondly, we have used the transmission mode PPG as well as the reflection mode PPG when measuring NPV. The transmission and reflection modes of PPG have been studied by others (Uretzky and Palti, 1971; Nijboer *et al.*, 1981), who reported that the pulsatile and non-pulsatile changes in intensity of light were found to be very similar whether measured by transmitted or reflected light. In our study, although the each absolute value of NPV is different due to the volume correction factor (k), CPT and WWI produced strong statistical relationships between transmission PPG measurements in the index finger, ΔlnNPVt_IF as a reference, and reflection measurements in the middle finger, ΔlnNPVr_MF (see figure 2 panel A). Taken together, these results support the view that the transmission mode PPG and the reflection mode PPG are indeed the comparable method to derive changes in NPV values.

Thirdly, of all of the possible methods for monitoring blood volume changes, such as air or water plethysmography, strain gauge plethysmography, or electrical impedance plethysmography, we have concentrated on the use of photoelectric plethysmography. Whilst PPG does not provide an absolute measure of blood volume it is simple and convenient to use as well as low in cost. More importantly, other methods, for example, strain gauge plethysmography, which is also known to be able to provide quantitative volume measures in limbs and digits (Whitney, 1953), would be difficult, if not impossible, to use for measurements in the ear. As we have shown, PPG can be applied quite simply for measurements at different regions of the ear, with the attachment of small and lightweight optical sources and detectors. Despite its simplicity our results show that PPG is a viable method for the assessment of peripheral perfusion at the ear.

Fourthly, regardless of age group, the same tendencies in the results were observed; not only the significant decrease in *ln*NPVr_ECB from the baseline values to the values obtained during the CPT (table 1 and table 3), but also the significant relationship during the CPT between ΔlnNPVt_IF as the reference and ΔlnNPVr_ECB are observed (table5), though the reactivities of each *ln*NPV during CPT in the middle-aged group is marginally smaller than those in the young group. These results mean that the pattern of the autonomic vascular control is very similar regardless of age. These findings provide more confidence in the NPVr_ECB as an alternative to NPVt_IF for evaluating physiological and psychological stress.

Finally, in our experiment subjects were required to remain still with the effect that their central blood oxygen levels were assumed to be approximately constant and so the absorptivity (ε) could also

be assumed to be essentially constant. This consideration is necessary when using the 940nm wavelength light source. However, the peripheral oxygen levels can be changed in various circumstances, especially during exercise and it would therefore be preferable to use an isosbestic wavelength, such as 805nm, to avoid this problem.

4.1. Limitation of this study

In this study, we have only used the cold pressor test and warm water immersion as challenges. To evaluate stress with NPV, however, there are several stress tasks that may initiate peripheral vasoconstriction, examples including mental arithmetic, mirror tracing, and social speech (Allen *et al.*, 1997; Matsumura *et al.*, 2011a; Matsumura *et al.*, 2012; Sawada *et al.*, 2001). Therefore, further laboratory studies using other kinds of stressors are needed to examine ear NPV more fully.

We have not yet shown that the ear NPV measurement performed with our methodology can actually be applied to the investigation of stresses experienced in normal daily life. This is because the stressful tasks used in laboratory experimentation do not necessarily reflect daily life situations, for example during working and sporting activities (Matsumura *et al.*, 2011b; Yamakoshi *et al.*, 2010). Therefore, further studies are also needed to examine ear NPV under normal daily life situations.

5. Conclusions

The normalized pulse volume derived from reflection mode PPG at the bottom of the ear-canal, NPVr_ECB, was found to be a valid measure when compared with reference measurements made in an index finger under cold pressor tests and warm water immersion to produce peripheral vasoconstriction. We conclude that NPVr_ECB might be suitable as a practical index for evaluating physiological and psychological stress in daily life.

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Table 1. Mean (S.D.) values of *ln*PV and $ln\boldsymbol{I}_{dc}$, as well as *ln*NPV for different anatomical positions in the 'young' group, during baseline and while performing the cold pressor test.

		Cold pressor test (CPT)		
	Baseline (5 min)	CPT1 (0 ~ 30 s)	CPT2 (30 ~ 60 s)	CPT3 (60 ~ 90 s)
Index finger				
*ln*PVt (mV)	2.57 (0.21)	1.69^{***} (0.26)	1.42^{***} (0.23)	1.55^{***} (0.27)
$ln\boldsymbol{I}_{dc}$t (mV)	5.38 (0.18)	5.66^{***} (0.19)	5.76^{***} (0.19)	5.76^{***} (0.20)
*ln*NPVt (a.u.)	−3.20 (0.26)	-4.40^{***} (0.26)	-4.77^{***} (0.31)	-4.63^{***} (0.38)
Middle finger				
*ln*PVr (mV)	2.62 (0.26)	1.33^{***} (0.31)	0.98^{***} (0.37)	1.15^{***} (0.42)
$ln\boldsymbol{I}_{dc}$r (mV)	8.39 (0.09)	8.41^{***} (0.09)	8.42^{***} (0.09)	8.42^{***} (0.09)
*ln*NPVr (a.u.)	−6.20 (0.23)	-7.55^{***} (0.36)	-7.85^{***} (0.41)	-7.68^{***} (0.45)
The top of the ear-canal				
*ln*PVr (mV)	0.54 (0.25)	0.36^{*} (0.35)	0.36^{*} (0.35)	0.41 (0.32)
$ln\boldsymbol{I}_{dc}$r (mV)	8.42 (0.08)	8.42^{*} (0.08)	8.42^{*} (0.08)	8.43^{*} (0.08)
*ln*NPVr (a.u.)	−8.29 (0.26)	−8.40 (0.31)	−8.46 (0.38)	−8.42 (0.38)
The bottom of the ear-canal				
*ln*PVr (mV)	0.80 (0.35)	0.62^{**} (0.37)	0.54^{***} (0.39)	0.57^{**} (0.39)
$ln\boldsymbol{I}_{dc}$r (mV)	8.39 (0.08)	8.39 (0.08)	8.39 (0.08)	8.39 (0.09)
*ln*NPVr (a.u.)	−8.01 (0.37)	-8.18^{**} (0.39)	-8.27^{***} (0.44)	-8.24^{**} (0.44)
The upper part of the ear-auricle				
*ln*PVr (mV)	−0.25 (0.22)	−0.24 (0.21)	−0.24 (0.16)	−0.13 (0.17)
$ln\boldsymbol{I}_{dc}$r (mV)	8.39 (0.07)	8.39 (0.07)	8.39 (0.07)	8.39 (0.07)
*ln*NPVr (a.u.)	−9.06 (0.18)	−9.06 (0.21)	−9.05 (0.16)	−8.90 (0.26)
The lower part of the ear-auricle				
*ln*PVr (mV)	0.29 (0.35)	0.18 (0.29)	0.12^{*} (0.26)	0.18 (0.23)
$ln\boldsymbol{I}_{dc}$r (mV)	8.40 (0.07)	8.40 (0.07)	8.40 (0.08)	8.40 (0.07)
*ln*NPVr (a.u.)	−8.52 (0.34)	−8.64 (0.29)	-8.70^{*} (0.28)	−8.64 (0.22)

Note. PV = pulse volume (gain = 150); $\boldsymbol{I}_{dc}$ = intensity of PPG signal (gain = 100); PPG = photoelectric plethysmography; NPV = normalized pulse volume; PVt, $\boldsymbol{I}_{dc}$t, NPVt = PV, $\boldsymbol{I}_{dc}$, and NPV using the transmission mode PPG, respectively; PVr, $\boldsymbol{I}_{dc}$r, NPVr = PV, $\boldsymbol{I}_{dc}$, and NPV using the reflection mode PPG, respectively; a.u. = arbitrary unit.

The comparisons between 5 min mean during baseline and CPT mean for each 30 s block by Ryan's method.
$^{*}p < 0.05$. $^{**}p < 0.01$. $^{***}p < 0.001$.

Table 2. Mean (S.D.) values of *ln*PV and $ln\boldsymbol{I}_{dc}$, as well as *ln*NPV for different anatomical positions in the 'young' group, during baseline and while performing the warm water immersion.

		Warm water immersion (WWI)		
	Baseline (5 min)	WWI1 (0 ~ 30 s)	WWI2 (30 ~ 60 s)	WWI3 (60 ~ 90 s)
Index finger				
*ln*PVt (mV)	2.57 (0.21)	2.34^{***} (0.20)	2.56 (0.26)	2.55 (0.23)
$ln\boldsymbol{I}_{dc}$t (mV)	5.38 (0.18)	5.56^{***} (0.19)	5.48^{**} (0.17)	5.47^{**} (0.19)
*ln*NPVt (a.u.)	−3.20 (0.26)	-3.64^{***} (0.23)	−3.32 (0.27)	−3.32 (0.31)
Middle finger				
*ln*PVr (mV)	2.62 (0.26)	2.14^{***} (0.27)	2.52 (0.38)	2.48 (0.38)
$ln\boldsymbol{I}_{dc}$r (mV)	8.39 (0.09)	8.40 (0.09)	8.39 (0.09)	8.39 (0.09)
*ln*NPVr (a.u.)	−6.20 (0.23)	-6.71^{***} (0.24)	−6.29 (0.35)	−6.34 (0.38)
The top of the ear-canal				
*ln*PVr (mV)	0.54 (0.25)	0.48 (0.29)	0.47 (0.27)	0.50 (0.25)
$ln\boldsymbol{I}_{dc}$r (mV)	8.42 (0.08)	8.42^{*} (0.08)	8.42^{*} (0.08)	8.42^{*} (0.08)
*ln*NPVr (a.u.)	−8.29 (0.26)	−8.36 (0.33)	−8.37 (0.31)	−8.33 (0.28)
The bottom of the ear-canal				
*ln*PVr (mV)	0.80 (0.35)	0.73 (0.37)	0.72 (0.38)	0.74 (0.36)
$ln\boldsymbol{I}_{dc}$r (mV)	8.39 (0.08)	8.39 (0.09)	8.39 (0.09)	8.39 (0.09)
*ln*NPVr (a.u.)	−8.01 (0.37)	−8.08 (0.41)	−8.08 (0.43)	−8.06 (0.41)
The upper part of the ear-auricle				
*ln*PVr (mV)	−0.25 (0.22)	−0.24 (0.20)	−0.31 (0.21)	−0.26 (0.19)
$ln\boldsymbol{I}_{dc}$r (mV)	8.39 (0.07)	8.39 (0.07)	8.39 (0.07)	8.39 (0.07)
*ln*NPVr (a.u.)	−9.06 (0.18)	−9.05 (0.19)	−9.12 (0.19)	−9.07 (0.17)
The lower part of the ear-auricle				
*ln*PVr (mV)	0.29 (0.35)	0.23 (0.35)	0.21 (0.32)	0.24 (0.31)
$ln\boldsymbol{I}_{dc}$r (mV)	8.40 (0.07)	8.40 (0.07)	8.40 (0.08)	8.40 (0.08)
*ln*NPVr (a.u.)	−8.52 (0.34)	−8.59 (0.36)	−8.61 (0.34)	−8.58 (0.34)

Note. For details, see the legend of table 1.

The comparisons between 5 min mean during baseline and WWI mean for each 30 s block by Ryan's method.
$^{*}p < 0.05$. $^{**}p < 0.01$. $^{***}p < 0.001$.

Table 3. Mean (S.D.) values of *ln*PV and $ln\boldsymbol{I}_{dc}$, as well as *ln*NPV for different anatomical positions in the 'middle-aged' group, during baseline and while performing the cold pressor test.

		Cold pressor test (CPT)		
	Baseline (5 min)	CPT1 (0 ~ 30 s)	CPT2 (30 ~ 60 s)	CPT3 (60 ~ 90 s)
Index finger				
*ln*PVt (mV)	1.87 (0.47)	1.03^{***} (0.35)	1.15^{***} (0.32)	1.40^{***} (0.43)
$ln\boldsymbol{I}_{dc}$t (mV)	5.03 (0.35)	5.26^{***} (0.33)	5.32^{***} (0.30)	5.29^{***} (0.32)
*ln*NPVt (a.u.)	−3.57 (0.27)	-4.64^{***} (0.28)	-4.58^{***} (0.23)	-4.30^{***} (0.35)
Middle finger				
*ln*PVr (mV)	2.60 (0.24)	1.45^{***} (0.16)	1.59^{***} (0.18)	1.86^{***} (0.26)
$ln\boldsymbol{I}_{dc}$r (mV)	8.44 (0.03)	8.47^{***} (0.02)	8.47^{***} (0.03)	8.46^{***} (0.03)
*ln*NPVr (a.u.)	−6.27 (0.23)	-7.45^{***} (0.13)	-7.29^{***} (0.20)	-7.02^{***} (0.27)
The top of the ear-canal				
*ln*PVr (mV)	0.40 (0.23)	0.33 (0.22)	0.37 (0.15)	0.45 (0.16)
$ln\boldsymbol{I}_{dc}$r (mV)	8.46 (0.04)	8.46 (0.04)	8.46 (0.04)	8.46 (0.04)
*ln*NPVr (a.u.)	−8.48 (0.26)	−8.55 (0.25)	−8.51 (0.18)	−8.43 (0.20)
The bottom of the ear-canal				
*ln*PVr (mV)	0.66 (0.35)	0.51 (0.42)	0.46^{*} (0.43)	0.59 (0.40)
$ln\boldsymbol{I}_{dc}$r (mV)	8.46 (0.02)	8.46 (0.02)	8.46 (0.02)	8.46 (0.03)
*ln*NPVr (a.u.)	−8.21 (0.36)	−8.36 (0.44)	-8.42^{*} (0.45)	−8.29 (0.43)
The upper part of the ear-auricle				
*ln*PVr (mV)	0.08 (0.27)	0.14 (0.15)	0.30 (0.24)	0.30 (0.29)
$ln\boldsymbol{I}_{dc}$r (mV)	8.44 (0.03)	8.44 (0.03)	8.43 (0.04)	8.43 (0.03)
*ln*NPVr (a.u.)	−8.78 (0.26)	−8.73 (0.14)	−8.56 (0.27)	−8.56 (0.30)
The lower part of the ear-auricle				
*ln*PVr (mV)	0.11 (0.30)	0.05 (0.29)	0.08 (0.31)	0.20 (0.17)
$ln\boldsymbol{I}_{dc}$r (mV)	8.43 (0.02)	8.43 (0.02)	8.43 (0.02)	8.43 (0.02)
*ln*NPVr (a.u.)	−8.75 (0.34)	−8.81 (0.30)	−8.78 (0.30)	−8.66 (0.21)

Note. For details, see the legend of table 1.

The comparisons between 5 min mean during baseline and CPT mean for each 30 s block by Ryan's method.
$^{*}p < 0.05$. $^{**}p < 0.01$. $^{***}p < 0.001$.

Table 4. Mean (S.D.) values of *ln*PV and $ln\boldsymbol{I}_{dc}$, as well as *ln*NPV for different anatomical positions in the 'middle-aged' group, during baseline and while performing the warm water immersion.

		Warm water immersion (WWI)		
	Baseline (5 min)	WWI1 (0 ~ 30 s)	WWI2 (30 ~ 60 s)	WWI3 (60 ~ 90 s)
Index finger				
*ln*PVt (mV)	1.87 (0.47)	1.47* (0.51)	1.69 (0.52)	1.74 (0.53)
$ln\boldsymbol{I}_{dc}$t (mV)	5.03 (0.35)	5.20* (0.42)	5.14 (0.46)	5.10 (0.47)
*ln*NPVt (a.u.)	−3.57 (0.27)	−4.15* (0.57)	−3.86 (0.68)	−3.77 (0.69)
Middle finger				
*ln*PVr (mV)	2.60 (0.24)	1.95** (0.52)	2.32 (0.64)	2.41 (0.63)
$ln\boldsymbol{I}_{dc}$r (mV)	8.44 (0.03)	8.46* (0.03)	8.45 (0.03)	8.45 (0.03)
*ln*NPVr (a.u.)	−6.27 (0.23)	−6.94** (0.53)	−6.54 (0.66)	−6.45 (0.65)
The top of the ear-canal				
*ln*PVr (mV)	0.40 (0.23)	0.35 (0.14)	0.30 (0.19)	0.35 (0.17)
$ln\boldsymbol{I}_{dc}$r (mV)	8.46 (0.04)	8.46 (0.04)	8.46 (0.04)	8.46 (0.04)
*ln*NPVr (a.u.)	−8.48 (0.26)	−8.54 (0.18)	−8.59 (0.21)	−8.54 (0.20)
The bottom of the ear-canal				
*ln*PVr (mV)	0.66 (0.35)	0.61 (0.30)	0.60 (0.34)	0.61 (0.31)
$ln\boldsymbol{I}_{dc}$r (mV)	8.46 (0.02)	8.45 (0.02)	8.45 (0.02)	8.45 (0.02)
*ln*NPVr (a.u.)	−8.21 (0.36)	−8.27 (0.32)	−8.27 (0.35)	−8.27 (0.33)
The upper part of the ear-auricle				
*ln*PVr (mV)	0.08 (0.27)	−0.01 (0.20)	−0.02 (0.22)	0.01 (0.24)
$ln\boldsymbol{I}_{dc}$r (mV)	8.44 (0.03)	8.44 (0.03)	8.44 (0.03)	8.44 (0.03)
*ln*NPVr (a.u.)	−8.78 (0.26)	−8.88 (0.18)	−8.88 (0.20)	−8.86 (0.21)
The lower part of the ear-auricle				
*ln*PVr (mV)	0.11 (0.30)	0.04 (0.24)	−0.05 (0.38)	0.02 (0.30)
$ln\boldsymbol{I}_{dc}$r (mV)	8.43 (0.02)	8.43 (0.03)	8.43 (0.03)	8.43 (0.03)
*ln*NPVr (a.u.)	−8.75 (0.30)	−8.82 (0.26)	−8.92 (0.41)	−8.84 (0.31)

Note. For details, see the legend of table 1.

The comparisons between 5 min mean during baseline and WWI mean for each 30 s block by Ryan's method.
$^{*}p < 0.05$. $^{**}p < 0.01$. $^{***}p < 0.001$.

Table 5. Pearson's correlation coefficient of Δ*ln*NPV from an index finger using transmission mode PPG as a reference with Δ*ln*PV and Δ*ln*NPV from the middle finger and the outer ear using reflection mode PPG in the 'young' and 'middle-aged' groups, respectively; during baseline and while performing cold and warm water immersion.

	Δ*ln*NPVt_IF in young[a]		Δ*ln*NPVt_IF in middle-aged[b]	
	CPT	WWI	CPT	WWI
Middle finger				
Δ*ln*PVr (mV)	–	–	–	–
Δ*ln*NPVr (a.u.)	0.90**	0.96**	0.80**	0.99**
The top of ear-canal				
Δ*ln*PVr (mV)	0.35	−0.12	0.13	0.15
Δ*ln*NPVr (a.u.)	0.45*	−0.10	0.14	0.17
The bottom of ear-canal				
Δ*ln*PVr (mV)	0.60**	0.17	0.59*	−0.17
Δ*ln*NPVr (a.u.)	0.62**	0.18	0.58*	−0.19
The upper part of the ear-auricle				
Δ*ln*PVr (mV)	0.18	−0.08	−0.03	−0.17
Δ*ln*NPVr (a.u.)	0.23	−0.09	−0.04	−0.18
The lower part of the ear-auricle				
Δ*ln*PVr (mV)	0.18	0.08	0.56*	−0.02
Δ*ln*NPVr (a.u.)	0.18	0.08	0.56*	0.10

Note. Δ*ln*NPVt_IF = *ln*NPVt reactivities (the difference between baseline and test values) from an index finger using the transmission mode PPG as a reference; Δ*ln*PVr = *ln*PVr reactivities using the reflection mode PPG; Δ*ln*NPVr = *ln*NPVr reactivities using the reflection mode PPG; CPT = cold pressor test; WWI = warm water immersion; a.u. = arbitrary unit.

[a]$n = 30$. [b]$n = 18$.

*$p < 0.05$. **$p < 0.01$.

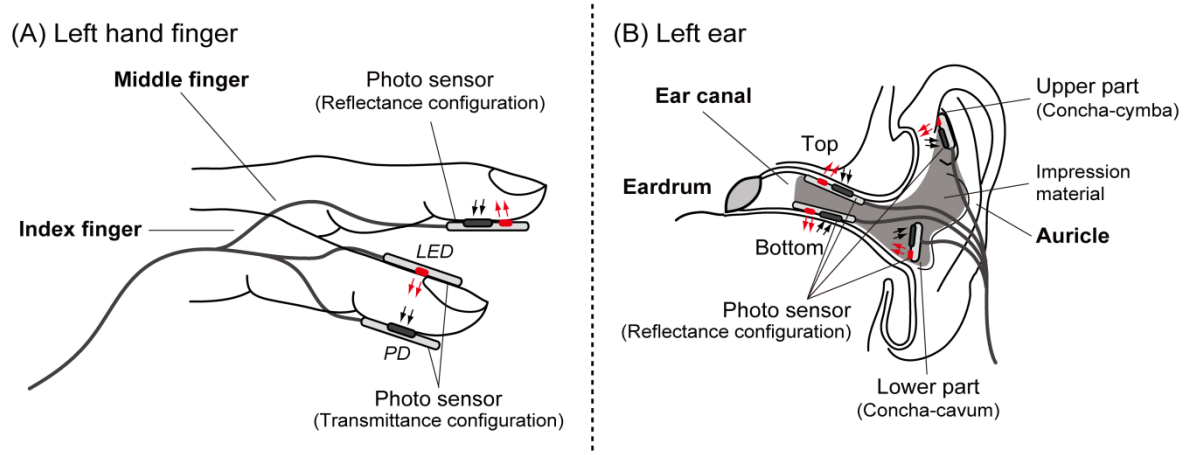

Figure 1. The placement of sensors for the measurement of NPV. Left hand finger; an index finger using the transmission type sensor as a reference; a middle finger using the reflection type sensor (panel A). Left ear; the top and bottom of the ear-canal, and the upper and lower part of the ear-auricle using the reflection type sensors (panel B).

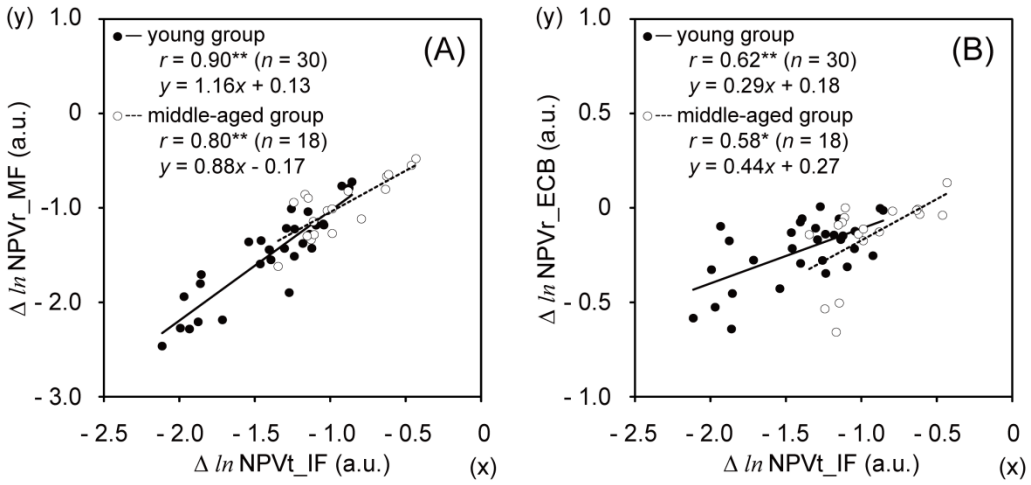

Figure 2. The scatter plot for Pearson's correlation analysis and the linear regression in the 'young' and 'middle-aged' groups between Δ*ln*NPV from an index finger using transmission mode PPG (Δ*ln*NPVt_IF) to x-axis and Δ*ln*NPV from the middle finger using reflection mode PPG (Δ*ln*NPVr_MF) to y-axis (panel A), and Δ*ln*NPV from the bottom of the ear-canal using reflection mode PPG (Δ*ln*NPVr_ECB) to y-axis (panel B). During baseline and while performing cold pressor test. Δ*ln*NPV is reactivity (the difference between baseline and test values) of *ln*NPV. Δ*ln*NPV is expressed in arbitrary units (a.u.). $^{*}p < 0.05$, $^{**}p < 0.01$.

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