**Non-technical Summary**

The arterial baroreflex is a closed-loop, negative feedback control system that senses baroreceptor pressure and controls systemic arterial pressure (AP) to attenuate perturbations in AP. The total arc of the baroreflex consists of two subsystems: the neural [baroreceptor pressure input to sympathetic nerve activity (SNA)] and peripheral (SNA input to AP) arcs. We show that although the spontaneous baroreflex transfer function obtained by closed-loop analysis has been believed to represent the neural arc function, it is inappropriate for system identification of the neural arc but is essentially appropriate for the peripheral arc under resting condition, when compared with open-loop transfer functions that have good predictabilities of time-series output dynamics from input signals. Our results indicate that in the spontaneous baroreflex system under closed-loop conditions, the peripheral arc (feedforward) function predominates over the neural arc (feedback) function, probably because of SNA component that is independent of the baroreceptor pressure input.

Word count: 149

**Abstract**

Although the dynamic characteristics of the baroreflex system have been described by baroreflex transfer functions obtained from open-loop analysis, the predictability of time-series output dynamics from input signals, which should confirm the accuracy of system identification, remains to be elucidated. Moreover, despite theoretical concerns over closed-loop system identification, the accuracy and the predictability of the closed-loop spontaneous baroreflex transfer function have not been evaluated compared with the open-loop transfer function. Using urethane and α-chloralose anesthetized, vagotomized and aortic-denervated rabbits (n=10), we identified open-loop baroreflex transfer functions by recording renal sympathetic nerve activity (SNA) while varying the vascularly isolated intracarotid sinus pressure (CSP) according to a binary random (white-noise) sequence (operating pressure ± 20 mmHg), and using a simplified equation to calculate closed-loop-spontaneous baroreflex transfer function while matching CSP with systemic arterial pressure (AP). Our results showed that the open-loop baroreflex transfer functions for the neural and peripheral arcs predicted the time-series SNA and AP outputs from measured CSP and SNA inputs, with $r^2$ of 0.8±0.1 and 0.8±0.1, respectively. In contrast, the closed-loop-spontaneous baroreflex transfer function for the neural arc was markedly different from the open-loop transfer function (enhanced gain increase and a phase lead), and did not predict the time-series SNA dynamics ($r^2$; 0.1±0.1). However, the closed-loop-spontaneous baroreflex transfer function of the peripheral arc partially matched the open-loop transfer function in gain and phase functions, and had limited but reasonable predictability of the time-series AP dynamics ($r^2$; 0.7±0.1). A numerical simulation suggested that a noise predominantly in the neural arc under resting condition might be a possible mechanism responsible for our findings. Furthermore, the predictabilities of the neural arc transfer functions obtained in open-loop and closed loop conditions were validated by closed-loop pharmacological (phenylephrine and nitroprusside infusions) pressure interventions. Time-series SNA responses to drug-induced AP changes predicted by the open-loop transfer function matched closely the measured responses ($r^2$; 0.9±0.1), whereas

SNA responses predicted by closed-loop-spontaneous transfer function deviated greatly and were the inverse of measured responses (r, -0.8 ± 0.2). These results indicate that although the spontaneous baroreflex transfer function obtained by closed-loop analysis has been believed to represent the neural arc function, it is inappropriate for system identification of the neural arc but is essentially appropriate for the peripheral arc under resting condition, when compared with open-loop analysis.

**Abbreviations**: AP, arterial pressure; SNA, sympathetic nerve activity; CSP, intra-carotid sinus pressure; r, linear correlation coefficient; RMS, root mean square

## INTRODUCTION

Arterial baroreflex plays a crucial role in circulatory control by its dynamic system characteristics (Eckberg & Sleight, 1992; Rowell, 1993). The baroreflex is a closed-loop, negative feedback control system that constantly senses arterial pressure (AP) by baroreceptors and quickly regulates systemic AP physiologically to attenuate perturbations in AP (Eckberg & Sleight, 1992; Rowell, 1993). The total arc baroreflex system consists of two subsystems: the neural and peripheral arcs (Kamiya *et al.*, 2005b; Kamiya *et al.*, 2008a; Kamiya *et al.*, 2010; Kawada *et al.*, 2010). The neural arc subsystem represents central processing from baroreceptor pressure to efferent sympathetic nerve activity (SNA), whereas the peripheral arc subsystem represents processing from SNA to systemic AP via peripheral circulatory organs including heart, kidney and blood vessels (Figure 1) (Ikeda *et al.*, 1996; Kamiya *et al.*, 2005b).

Transfer function analysis is a powerful tool to determine the dynamic characteristics of biosystems. This analysis has revealed the dynamic causality mainly in "open-loop" biosystems, including cerebral autoregulation (Zhang *et al.*, 2002), renal vascular function (DiBona & Sawin, 2003, 2004), heart-rate control (Ikeda *et al.*, 1995) and cutaneous circulation (Kamiya *et al.*, 2008b). We have applied the transfer function analysis to characterize the "closed-loop" arterial baroreflex system, in which we used the open-loop and white-noise pressure perturbation techniques to overcome the difficulties of closed-loop system identification (see APPENDIX A) (Ikeda *et al.*, 1996; Kawada *et al.*, 2002; Kamiya *et al.*, 2005b; Kamiya *et al.*, 2008a). We have reported that the neural arc transfer function ($H_n$) has derivative and high-cut filter characteristics with a pure delay, indicating that more rapid change of arterial pressure results in greater response of SNA to pressure change (Kawada *et al.*, 2002; Kamiya *et al.*, 2005b); whereas the peripheral arc transfer function ($H_p$) has second-order low-pass filter characteristics with a pure delay (see APPENDIX B) (Kawada *et al.*, 2002; Kamiya *et al.*, 2005b). However, at least two important issues remain to be elucidated.

First, a hallmark of the transfer function, the predictability of time-series output

dynamics from input signals (Ikeda *et al.*, 1995; Kamiya *et al.*, 2008b), has not yet been investigated in the baroreflex system. Accurate system identification of the transfer function yields good predictability, whereas inappropriate system identification results in poor predictability. In the present study, we tested the first hypothesis that the open-loop baroreflex transfer functions of the neural and peripheral arcs are capable of predicting time-series SNA and AP output dynamics from baroreceptor pressure and SNA inputs, respectively.

Second, identifying transfer functions is theoretically difficult under closed-loop and spontaneous resting baroreflex conditions. The reason is that unknown noises in the neural and peripheral arcs would interfere with the accuracy of system identification in closed-loop-spontaneous conditions, in contrast to open-loop transfer function identification where the interfering effects of noises would be eliminated by the open-loop and white-noise pressure perturbation techniques (Ikeda *et al.*, 1996; Kawada *et al.*, 2002; Kamiya *et al.*, 2005b; Kamiya *et al.*, 2008a) (see APPENDIX A). Although earlier interesting studies have applied a simplified (open-loop–like) calculation of transfer function to closed-loop-spontaneous resting baroreflex condition in humans (Cooke *et al.*, 1999; Cooke *et al.*, 2009; Ogoh *et al.*, 2009) and animals (Orea *et al.*, 2007) without opening the loop, whether the reported transfer functions are actually capable of predicting time-series output dynamics has not been verified. In addition, the accuracy and limitation of closed-loop-spontaneous baroreflex transfer functions remain unclear from the viewpoint of comparing with open-loop transfer functions. In the present study, we tested the second hypothesis that the closed-loop-spontaneous baroreflex transfer function is limited to predict baroreflex dynamics compared with the open-loop transfer function.

In the present study, by artificially controlling intra-carotid sinus pressure (CSP) and recording renal SNA and systemic AP, we identified the open-loop baroreflex transfer functions by introducing CSP perturbation according to a binary random (white-noise) sequence. We also determined the closed-loop-spontaneous baroreflex transfer functions by matching CSP with systemic AP. We then compared the characteristics and predictability of

these transfer functions. Our results confirmed good predictability of the open-loop baroreflex transfer functions, and unexpectedly indicated that the closed-loop-spontaneous transfer function approximately matched the open-loop transfer function for the peripheral arc but deviated markedly from the open-loop transfer function for the neural arc. Thus, the closed-loop-spontaneous baroreflex transfer function is inappropriate for system identification of the neural arc but is partially appropriate for the peripheral arc under resting condition, compared with the open-loop analysis. These findings may have great impact, because the closed-loop spontaneous baroreflex transfer function has been believed to represent the neural arc function (Orea *et al.*, 2007; Cooke *et al.*, 2009; Ogoh *et al.*, 2009).

## MATERIAL AND METHODS

### Animal preparation

Animals were cared for in strict accordance with the Guiding Principles for the Care and Use of Animals in the Field of Physiological Science approved by the Physiological Society of Japan and the National Cerebral and Cardiovascular Center Research Institute. All experiments complied with the policies and regulations of the Journal of Physiology given by Drummond (Drummond, 2009). Ten Japanese white rabbits weighing 2.4-3.3 kg were initially anaesthetized by intravenous injection (2 ml $kg^{-1}$) of a mixture of urethane (250 mg/ml) and α-chloralose (40 mg/ml). Anaesthesia was maintained by continuously infusing the anaesthetics at a rate of 0.33 ml $kg^{-1}$ $h^{-1}$ using a syringe pump (CFV-3200, Nihon Kohden, Tokyo). The rabbits were mechanically ventilated with oxygen-enriched room air. Bilateral carotid sinuses were isolated vascularly from the systemic circulation by ligating the internal and external carotid arteries and other small branches originating from the carotid sinus regions. The isolated carotid sinuses were filled with warmed physiological saline pre-equilibrated with atmospheric air, through catheters inserted via the common carotid arteries. CSP was controlled by a servo-controlled piston pump (model ET-126A, Labworks; Costa Mesa, CA). Bilateral vagal and aortic depressor nerves were sectioned in the middle of the neck region to eliminate reflexes from the cardiopulmonary region and the aortic arch.

Systemic AP was measured using a high-fidelity pressure transducer (Millar Instruments; Houston, TX) inserted retrograde from the right common carotid artery below the isolated carotid sinus region. A catheter was inserted into the right femoral vein to infuse phenylephrine and nitroprusside. Body temperature was maintained at around 38°C with a heating pad.

The left renal sympathetic nerve was exposed retroperitoneally. A pair of stainless steel wire electrodes (Bioflex wire AS633, Cooner Wire) was attached to the nerve to record renal SNA. The nerve fibres peripheral to the electrodes were ligated tightly and crushed to eliminate afferent signals. The nerve and electrodes were covered with a mixture of silicone gel (Silicon Low Viscosity, KWIK-SIL, World Precision Instrument, Inc., FL) to insulate and immobilize the electrodes. The preamplified SNA signal was band-pass filtered at 150-1,000 Hz. These nerve signals were full-wave rectified and low-pass filtered with a cutoff frequency of 30 Hz to quantify the nerve activity.

**Protocols**

After the surgical preparation, all animals (n = 10) were maintained supine. The overall scheme of the experimental design is shown in Figure 2. Protocols 1-4 were conducted in randomized order at intervals of at least 5 min, while protocol 5 was done finally. In all protocols, bilateral CSP was controlled by a servo-controlled piston pump (Kawada *et al.*, 2002). The SNA, CSP and AP were recorded at a sampling rate of 200 Hz using a 12-bit analog-to-digital converter. Data were stored on the hard disk of a dedicated laboratory computer system.

Before these protocols, operating AP and SNA in baroreflex closed-loop condition were determined. First, CSP was matched with systemic AP to close the baroreflex loop. After at least 5 min of stabilization, the variables were recorded for 10 min, and the average AP over 10 min was defined as the operating AP under closed-loop condition.

*System identification studies*

Protocol 1 was performed to identify the open-loop baroreflex transfer functions.

After at least 5 minutes of stabilization, CSP was randomly assigned at 20 mmHg above or below the operating AP every 500 ms according to a binary random (white-noise) sequence, in which the input power spectrum of CSP was reasonably flat up to 1 Hz (Kawada *et al.*, 2002). The variables were recorded for 10 min and stored for analysis.

Protocol 2 was performed to determine the closed-loop-spontaneous baroreflex transfer functions by a convenient method of applying the same calculation as that used in open-loop condition of protocol 1 (see APPENDIX A). CSP was matched with systemic AP to close the baroreflex loop. After at least 5 min of stabilization, the variables were recorded for 10 min and stored for analysis.

*Predictability studies*

Protocol 3, 4 and 5 were performed to investigate the predictability of baroreflex transfer functions. In protocol 3 (open loop), CSP was randomly assigned at 20 mmHg above or below the operating AP. The variables were recorded for 10 min and stored for analysis.

In protocol 4 (closed loop), CSP was matched with systemic AP to close the baroreflex loop. After at least 5 minutes of stabilization, the variables were recorded for at least 10 min and stored for analysis.

Protocol 5 was also performed to investigate the predictability of baroreflex transfer functions during sequential pharmacological pressure interventions in closed-loop condition. CSP was matched with systemic AP. After at least 2 min of stabilization, phenylephrine hydrochloride (3 μg/kg) was bolus infused through a venous catheter inserted into the right femoral vein, followed 1-2 min later by sodium nitroprusside (4 μg/kg) and then 1-2 min later by the second phenylephrine hydrochloride infusion (4 μg/kg). The variables were recorded continuously for at least 10-11 min and stored for analysis.

**Data analysis**

SNA signal was normalized by the following steps. First, 0 arbitrary unit (a.u.) was assigned to the post-mortem noise level. Second, 100 a.u. was assigned to the SNA signals averaged over 10 min before protocols. Lastly, the other SNA signals in protocols 1-5 were

then normalized to these values.

In protocol 1, we calculated the open-loop transfer (gain and phase) and coherence functions from CSP input to SNA in the neural arc ($H_{n\text{-}open}$) and from SNA to AP in the peripheral arc ($H_{p\text{-}open}$). We resampled CSP and SNA at 10 Hz and segmented them into 10 sets of 50% overlapping bins of $2^{10}$ data point each. The segment length was 102.4 s, which yielded the lowest frequency bound of 0.01 (0.0097) Hz. We subtracted a linear trend and applied a Hanning window for each segment. We then performed fast Fourier transform to obtain frequency spectra of input (x) and output (y). The inputs are CSP and SNA, while the outputs are SNA and AP in the neural and peripheral arc subsystems, respectively. We ensemble averaged the input power [Sxx(f)], output power [Syy(f)], and cross power between input and output [Syx(f)] over the 10 segments. Then, we calculated the transfer function [H(f)] from input to output as follows:

$$H(f) = \frac{Syx(f)}{Sxx(f)} \quad \text{----- (1)}$$

Although individual noise may be present in the neural and peripheral arc subsystems, the effects of noise on the calculations of transfer functions are eliminated by open-loop operation and white-noise–like perturbation of CSP (see APPENDIX A, Figure 1A).

To quantify the linear dependence between input and output in the frequency domain, we calculated the magnitude-squared coherence function [Coh(f)] as follows:

$$Coh(f) = \frac{|Syx(f)|^2}{Sxx(f)Syy(f)} \quad \text{----- (2)}$$

The coherence values range from zero to unity. Unity coherence indicates a perfect linear dependence between input and output, whereas zero coherence indicates total independence of these two signals. To quantify the errors on individual gain and phase estimates, we calculated the normalized random error [ε(f)] as follows:

$$\varepsilon(f) = \sqrt{\frac{1 - coh(f)}{2n_d coh(f)}} \quad \text{----- (3)}$$

where $n_d$ is a number of distinct subrecord, when the error in gain factor estimate matches that in phase factor estimate (Julius & Allan, 2000).

To quantify the transfer characteristics in the time domain, step response was calculated by discrete from convolution integral as follows:

$$Y(t) = \sum_{\tau=0}^{N} h(\tau) \cdot X(t-\tau) \quad \text{----- (4)}$$

where $h(\tau)$ is the impulse response obtained by inverse fast Fourier transform of the transfer function [H(f)]; $N$ is the total number of data elements; $\tau$ is the convolution parameter; $t$ is time in increments of 0.1 s (or 10 Hz); $X(t)=0$ for $t<0$ and $X(t)=1$ for $t \geq 0$.

It should be noted that since protocol 2 was a closed-loop and spontaneous baroreflex condition, unknown noise, if present in the neural and peripheral arc subsystems, would affect the accuracy of system identification (see APPENDIX A, Figure 1B). Based on earlier studies (Cooke *et al.*, 1999; Cooke *et al.*, 2009; Ogoh *et al.*, 2009), we applied a simplified (open-loop–like) calculation of transfer function to the closed-loop spontaneous resting baroreflex condition, and estimated the closed-loop-spontaneous baroreflex transfer functions from AP input to SNA in the neural arc ($H_{n\text{-closed-spon}}$) and from SNA to AP in the peripheral arc ($H_{p\text{-closed-spon}}$), together with coherence functions and step responses (see APPENDIX A).

In protocols 3 and 4, we calculated the predicted time-series output dynamics (SNA and AP) from measured input signals (CSP/AP and SNA in the neural and peripheral arc, respectively), using Eq. (4) and impulse response obtained from the transfer functions in protocols 1 and 2. The predicted output was scatter plotted, and compared with the actually measured output by calculating the linear correlation coefficient (r) and root mean square (RMS). The analysis was performed using the data at arbitrarily selected 1 and 3 min in protocols 3 and 4, respectively.

In protocols 5, similar to protocol 3 and 4, we calculated the predicted time-series output dynamics of SNA from measured pressure input signals (CSP/AP in the neural arc) during pharmacological interventions. The predicted SNA was scatter-plotted, and compared

with the actual SNA measurements by calculating r and RMS. The analysis was performed using the data for 10-11 min. Since AP was determined by interventions (phenylephrine and nitroprusside infusions) and not by SNA, we did not calculate the predicted AP dynamics from the measured SNA signals.

**Statistic analysis**

All data are presented as means ± S.D. Paired t-test and repeated measures analysis of variance with post hoc multiple comparisons were used to compare variables as appropriate. Differences were considered significant when $P<0.05$.

## RESULTS

**Open-loop transfer function (protocol 2)**

Figure 3 shows a typical example of the open-loop system identification of baroreflex transfer functions in protocol 2. CSP was perturbed according to a binary random (white-noise) sequence at 500-ms intervals (Figure 3A, green line). When CSP was increased, SNA decreased, and vice versa. In the frequency domain, the input power spectrum of CSP was reasonably flat up to 1 Hz (Figure 3B, green line).

The open-loop transfer function of the neural arc from CSP input to SNA ($H_{n\text{-}open}$; Figure 3C, left panels) showed that the gain increased as the frequency of CSP perturbation increased between 0.01 Hz and 0.4 Hz, indicating dynamic high-pass characteristics. The phase approached $-\pi$ at the lowest frequency, indicating a negative SNA response to CSP changes, and lagged as the frequency increased (Figure 3C, left panels). The coherence was over 0.8 between 0.03 to 0.4 Hz except at around 0.35 Hz (Figure 3C, left panels). The step response (Figure 3D, left panel) of SNA in response to CSP consisted of an initial decrease followed by partial recovery and then steady-state.

The open-loop transfer function of peripheral arc from SNA input to AP ($H_{p\text{-}open}$, Figure 3C, right panels) showed that the gain decreased as the frequency increased, indicating low-pass characteristics. The phase approached zero at the lowest frequency,

indicating a positive AP response to SNA changes, and lagged as the frequency increased (Figure 3C, right panels). The coherence was over 0.8 between 0.01 to 0.3 Hz except at around 0.2 Hz (Figure 3C, right panels). The step response (Figure 3D, right panel) of SNA to CSP was a gradual increase to steady-state.

The transfer function of baroreflex total arc from CSP input to systemic AP identified in open-loop condition (Figure 3E) showed that the gain decreased as the frequency increased, indicating low-pass characteristics that were milder than $H_{p\text{-}open}$. The phase approached $-\pi$ at the lowest frequency, indicating negative feedback system characteristics of baroreflex (negative AP response to CSP changes). The phase lagged as frequency increased. The transfer function of total arc was almost consistent with multiplication of tandemly arranged open-loop transfer functions of neural ($H_{n\text{-}open}$) and peripheral ($H_{p\text{-}open}$) arcs (Figures 1A and 1B), at the frequency where their coherence functions were high.

**Closed-loop-spontaneous transfer function (protocol 3)**

Figure 4 shows a typical example of the closed-loop-spontaneous transfer functions simplified calculated in protocol 3 by applying open-loop–like calculations to closed-loop spontaneous data. The data were obtained from the same animal as in Figure 3.

We closed the baroreflex loop by matching CSP with systemic AP. The exact match of the two parameters was demonstrated by autospectrum (Figure 4B) and beat-to-beat waveform (Figure 4C); both showing overlapping of CSP (green line) and systemic AP (black line). The exact match was further confirmed by the transfer functions from CSP to systemic AP (Figure 4D), which showed that the gain, phase and coherence functions were maintained constant at 1, zero and 1, respectively.

The closed-loop-spontaneous transfer function of the neural arc ($H_{n\text{-}closed\text{-}spon}$) from CSP (that equalled AP) to SNA (Figure 4E, left panels, black line) was markedly different from the open-loop transfer function ($H_{n\text{-}open}$, red line) with respect to gain, phase, coherence and step response. The increase in gain versus frequency was steeper; the gain was thus higher and the coherence was lower in $H_{n\text{-}closed\text{-}spon}$ compared with $H_{n\text{-}open}$. The phase led as

frequency increased, while the step response oscillated (Figure 4F, left panel) in $H_{n\text{-closed-spon}}$, which were markedly different from $H_{n\text{-open}}$.

In contrast to the neural arc, the closed-loop-spontaneous transfer function for the peripheral arc ($H_{p\text{-closed-spon}}$) from SNA to AP (Figure 4E, right panels, black line) approximated that of the open-loop transfer function ($H_{p\text{-open}}$, red line). The gain (except at 0.02-0.05 Hz) and phase were similar up to 0.3 Hz, although the coherence was lower in $H_{p\text{-closed-spon}}$ than in $H_{p\text{-open}}$ (common feature for both neural and peripheral arcs). The step response was similar to that of $H_{p\text{-open}}$ except a slower time constant (Figure 4F, right panel). Because of the closed-loop condition, the gain and phase functions of $H_{p\text{-closed-spon}}$ were the inverse of those of $H_{n\text{-closed-spon}}$.

Since CSP exactly matched systemic AP in this closed-loop-spontaneous baroreflex condition, the transfer function of total arc baroreflex from CSP input to systemic AP was calculated as all-pass filter without modulating phase (Figure 4D). This is greatly different from the transfer function of total arc identified from open-loop experiments (Figure 3E).

**Comparison between open-loop and closed-loop-spontaneous transfer functions**

The closed-loop-spontaneous transfer functions (Figure 5A, blue lines) ($H_{n\text{-closed-spon}}$ and $H_{p\text{-closed-spon}}$) obtained from all animals (n=10) in protocol 2 were compared with the open-loop transfer functions (Figure 5A, red lines) in protocol 1. The step response was also compared between closed-loop-spontaneous (Figure 5B, blue line) and open-loop experiments (Figure 5B, red line).

In the neural arc (Figure 5A and 5B, left panels; Table 1), closed-loop-spontaneous transfer functions ($H_{n\text{-closed-spon}}$, blue lines) was markedly different from open-loop transfer functions ($H_{n\text{-open}}$, red lines), similar to the example shown in Figure 4E. The difference was characterized by an enhanced increase of gain versus frequency (slope), a phase lead, and an oscillation of step response. In contrast, in the peripheral arc (Figure 5A and 5B, right panels; Table 2), closed-loop-spontaneous transfer functions ($H_{p\text{-closed-spon}}$) was similar to open-loop transfer functions ($H_{n\text{-open}}$) in gain, phase and step response.

The transfer function of baroreflex total arc from CSP input to systemic AP in open-loop condition was identified as having low-pass filter characteristics with negative feedback in all animals. In contrast, the total arc transfer function in closed-loop-spontaneous condition had all-pass filter characteristics without modulating phase in all animals.

**Predictability of open-loop and closed-loop-spontaneous transfer function compared with data measured in open-loop condition (protocol 3)**

The ability of the neural arc transfer functions (determined by protocols 1 and 2) to predict output dynamics (SNA) from given input signals (CSP) in open-loop condition was quantified by comparing with the actual measurements of SNA response to CSP changes in protocol 3. Figure 6 shows a typical example obtained from the same animal as in Figures 1 and 2. CSP was randomly assigned at 20 mmHg above or below the operating AP (changes according to binary random (white-noise) sequence; Figure 6A, top panel). The SNA response to CSP changes predicted by the open-loop transfer function ($H_{n\text{-}open}$) (Figure 6A; third panel) resembled the actually measured SNA (Figure 6A, second panel) in both the timing (phase) and magnitude of neural burst. In contrast, the SNA response predicted by the closed-loop-spontaneous transfer function ($H_{n\text{-}closed\text{-}spon}$) (Figure 6A, bottom panel) was different from the actually measured SNA (Figure 6A, second panel), showing markedly exaggerated fluctuation and inconsistent burst timing. As a result, scatter plot analyses showed that the SNA predicted by $H_{n\text{-}open}$ correlated significantly with the actually measured SNA ($r^2$, 0.83; RMS, 13 au; $p < 0.05$) (Figure 6B), whereas the SNA predicted by $H_{n\text{-}closed\text{-}spon}$ showed no correlation ($r^2$, 0.04; RMS, 109 au) (Figure 6C). Using the data from all animals, the SNA predicted by $H_{n\text{-}open}$ correlated with the measured SNA ($r^2$, 0.8 ± 0.1; RMS, 15 ± 4 au; $p < 0.05$), whereas SNA predicted by $H_{n\text{-}closed\text{-}spon}$ showed no correlation ($r^2$, 0.1 ± 0.1; RMS, 102 ± 24 au).

In addition, the ability of the peripheral arc transfer functions (determined by protocols 1 and 2) to predict output dynamics (AP) from given input signals (SNA) in

open-loop condition was quantified by comparing with the actual measurements of AP response to SNA changes. Figure 7 shows an example obtained from the same animal as in Figure 6. The AP response to SNA predicted by the open-loop transfer function ($H_{p\text{-}open}$) (third panel) resembled closely the actually measured AP (second panel). The AP response (bottom panels) predicted by the closed-loop-spontaneous transfer function ($H_{p\text{-}closed\text{-}spon}$) was also similar but with limitations. Scatter plot analyses showed that the AP predicted by $H_{p\text{-}open}$ correlated well with the measured AP ($r^2$, 0.75; RMS, 2 mmHg; $p < 0.05$) (Figure 7B), whereas the AP predicted by $H_{p\text{-}closed\text{-}spon}$ correlated partially with the measured values ($r^2$, 0.58; RMS, 3 mmHg; $p < 0.05$) (Figure 7C). Using the data from all animals, the $H_{p\text{-}open}$-predicted AP correlated well with the measured AP ($r^2$, 0.8 ± 0.1; RMS, 2 ± 2 mmHg; $p < 0.05$). The $H_{p\text{-}closed\text{-}spon}$-predicted AP similarly correlated with the measured AP, but with lower $r^2$ (0.7 ± 0.1) and higher RMS (3 ± 3 mmHg) compared with $H_{p\text{-}open}$ ($p<0.05$).

**Predictability of open-loop and closed-loop-spontaneous transfer function compared with data measured in closed-loop-spontaneous condition (protocol 4)**

The ability of neural arc transfer functions (determined by protocols 1 and 2) to predict SNA from CSP input in closed-loop-spontaneous condition was quantified by comparing with the actual SNA measurements in protocol 4. Figure 8 shows a typical example. Since CSP was matched with systemic AP, spontaneous fluctuation of AP was observed (Figure 8A, top panel). AP increased spontaneously during 60-90 s but SNA did not decrease but increase, indicating that AP changes did not induce a negative SNA response via the baroreflex neural arc (Figure 8A, third panel). Indeed, SNA predicted by $H_{n\text{-}open}$ (Figure 8A, fourth panel) and $H_{n\text{-}closed\text{-}spon}$ (Figure 8A, bottom panel) were markedly different from the measured SNA (Figure 8A, second and third panels). Scatter plot analyses indicated that these predicted SNAs did not correlate with the measured SNA (Figures 8B and 8C).

In addition, the ability of peripheral arc transfer functions (determined by protocols 1 and 2) to predict AP from SNA input in closed-loop-spontaneous condition was also

quantified by comparing with the actually measured AP. Figure 9 shows a typical example obtained from the same animal as in Figure 8. The spontaneous changes in SNA (Figure 9A, second panel) appeared to precede those in AP (Figure 9A, third panel), suggesting that SNA changes induced a positive response of AP via the baroreflex peripheral arc. Indeed, the AP (gray lines, resampled at 0.1 Hz) predicted by $H_{p\text{-}open}$ (Figure 9A, fourth panel) and that predicted by $H_{p\text{-}closed\text{-}spon}$ (Figure 9A, bottom panel) resembled the measured AP (Figure 9A, third panel). Scatter plot analyses indicated that these predicted APs correlated well with the measured AP (Figures 9B and 9C).

Similar results were found in all animals in closed-loop-spontaneous resting condition. Changes in SNA always preceded alterations in AP, and induced a positive AP response. The closed-loop peripheral arc transfer function predicted the time series of AP dynamics with some degree of accuracy, whereas the neural arc transfer function failed to predict SNA.

**Predictability of open-loop and closed-loop-spontaneous transfer function compared with data measured during pharmacological pressure intervention in closed-loop condition (protocol 5)**

The ability of neural arc transfer functions (determined by protocols 1 and 2) to predict SNA from CSP change induced by pharmacological intervention under closed-loop condition was quantified by comparing with the actual SNA measurements in protocol 5. The intervention was sequential bolus infusions of phenylephrine-nitroprusside-phenylephrine. Figure 10 shows a typical example. CSP was matched with systemic AP (Figure 10A, first and second panels). The phenylephrine-nitroprusside-phenylephrine bolus infusions produced increase-decrease-increase and recovery changes in AP, which led to changes in SNA as follows. When AP (=CSP) increased, actually measured SNA decreased, and vice versa (Figure 10A, third panel).

The SNA response to CSP changes predicted by the open-loop transfer function ($H_{n\text{-}open}$) (Figure 10A; fourth panel) resembled the actually measured SNA (Figure 10A, third

panel) in both the timing (phase) and intensity of neural activity. In contrast, the SNA response predicted by the closed-loop-spontaneous transfer function ($H_{n\text{-}closed\text{-}spon}$) (Figure 10A, bottom panel) showed oppositely directed neural response as compared with actually measured SNA (Figure 10A, third panel). When AP (=CSP) increased, the predicted SNA increased whereas the measured SNA decreased, and vice versa. As a result, scatter plot analyses showed that the SNA predicted by $H_{n\text{-}open}$ correlated significantly with the actually measured SNA ($r^2$, 0.87; RMS, 17 au; $p < 0.05$) (Figure 10B), indicating a good predictability. However, the SNA predicted by $H_{n\text{-}closed\text{-}spon}$ showed negative correlation (r, -0.91; RMS, 114 au) (Figure 10C), indicating a lack of predictability. The relationship between CSP (=AP) and actually measured SNA (Figure 10D and 10E, open circles) was similar to that between CSP (=AP) and SNA predicted by $H_{n\text{-}open}$ (Figure 10D, red circles) but different from that between CSP and SNA predicted by $H_{n\text{-}closed\text{-}spon}$ (Figure 10E, blue circles). Using the data from all animals, the SNA predicted by $H_{n\text{-}open}$ correlated with the measured SNA ($r^2$, 0.9 ± 0.1; RMS, 16 ± 4 au; $p < 0.05$), whereas SNA predicted by $H_{n\text{-}closed\text{-}spon}$ showed negative correlation (r, -0.8 ± 0.1; RMS, 112 ± 35 au).

## DISCUSSION

### Good predictability of open-loop baroreflex transfer functions

Although baroreflex transfer functions have been identified by open-loop analysis (Ikeda *et al.*, 1996; Kawada *et al.*, 2002; Kamiya *et al.*, 2005b; Kamiya *et al.*, 2008a), whether the functions predict time-series output dynamics, which would confirm the accuracy of system identification of transfer function, remains to be elucidated. In the present study, we showed that the open-loop baroreflex transfer functions were able to predict output dynamics with high accuracy, even though the data set for determining the transfer functions (protocol 1) were different from that for investigating predictability (protocols 3, 4 and 5). The neural arc transfer function determined by open-loop experiment ($H_{n\text{-}open}$) predicted SNA responses to measured CSP changes with $r^2$ of 0.8 ± 0.1. Likewise, the peripheral arc transfer function

($H_{p\text{-}open}$) also predicted the AP responses to measured SNA changes with $r^2$ of 0.8 ± 0.1 (Figure 7). These results supported our first hypothesis that the open-loop baroreflex transfer functions for the neural and peripheral arcs are able to predict time-series SNA and AP outputs from baroreceptor pressure and SNA inputs, respectively. The good predictability indicates the accuracy of system identification of these transfer functions determined by open-loop experiments.

**Inappropriate system identification and limited predictability of closed-loop spontaneous baroreflex transfer functions**

Regarding the neural arc, our results showed that the transfer function determined under closed-loop-spontaneous conditions ($H_{n\text{-}closed\text{-}spon}$) was markedly different from that determined under open-loop conditions ($H_{n\text{-}open}$) (Figure 5). In $H_{n\text{-}closed\text{-}spon}$, the increase in gain versus frequency was markedly enhanced (enhanced high-pass filter). A phase lead rather than phase lag indicates that the calculated phase may be incorrect since $H_{n\text{-}open}$ showed a linear phase lag, reflecting a fixed pure time delay from baroreceptor pressure to SNA (Orea *et al.*, 2007). Furthermore, the step response of SNA to CSP predicted by $H_{n\text{-}closed\text{-}spon}$ oscillated, although an initial decrease followed by partial recovery was predicted by $H_{n\text{-}open}$ (Figure 5). These contradicting and strange characteristics of $H_{n\text{-}closed\text{-}spon}$ were associated with less appropriate predictability of time-series SNA output dynamics. Although the SNA predicted by $H_{n\text{-}open}$ in response to the measured changes in CSP was roughly similar to the actually measured SNA with respect to both amplitude and timing of neural burst (Figure 6A and 6B), the SNA predicted by $H_{n\text{-}closed\text{-}spon}$ was greatly different from the measured SNA, showing increased amplitude and inconsistent timing of neural burst (Figures 6A and 6C). Therefore, with regard the neural arc, these results support our second hypothesis that the closed-loop-spontaneous baroreflex transfer function is limited in its ability to predict the baroreflex dynamics compared with the open-loop transfer function.

Regarding the peripheral arc, however, the present study showed unexpected results. In contrast to the neural arc, the closed-loop-spontaneous baroreflex transfer

function for the peripheral arc ($H_{p\text{-}closed\text{-}spon}$) approximated the open-loop transfer function ($H_{p\text{-}open}$) not only in gain and phase functions but also in step response (Figure 5). The similar characteristics of $H_{p\text{-}closed\text{-}spon}$ and $H_{p\text{-}open}$ yielded high predictability of time-series AP dynamics. The AP predicted by $H_{p\text{-}closed\text{-}spon}$ in response to the measured SNA changes was roughly the same as the actually measured AP (Figure 7), although the correlation with measured data was lower than the AP predicted by $H_{p\text{-}open}$. Therefore, regarding the peripheral arc, these results support our second hypothesis and indicate that the closed-loop-spontaneous baroreflex transfer function is partially appropriate for system identification of the peripheral arc and is able to predict the time-series AP dynamics under resting condition despite slightly limited accuracy compared with the open-loop transfer function. This finding may have great impact, because the closed-loop spontaneous baroreflex transfer function has been believed to represent the neural arc function (Orea *et al.*, 2007; Cooke *et al.*, 2009; Ogoh *et al.*, 2009).

**Potential mechanism for the limitation of closed-loop spontaneous baroreflex transfer functions**

It is indeed difficult to understand why the closed-loop-spontaneous transfer function is inappropriate for system identification of the neural arc but is partially appropriate for the peripheral arc. As a possible mechanism, we examined the effects of noise on the calculation of the closed-loop-spontaneous transfer function using numerical simulations (Figure 11). The noise is considered as factors that modulate output dynamics independent of input. Figure 11 (upper panels in B, C and D) shows block diagrams of the closed-loop baroreflex system. $H_n$ represents central processing from baroreceptor pressure input to SNA, while $H_p$ represents peripheral processing from SNA input to systemic AP. According to our previous studies (Ikeda *et al.*, 1996; Kawada *et al.*, 2002), we modelled $H_n$ using derivative and high-cut filter characteristics with a pure delay, and $H_p$ using second-order low-pass filter with a pure delay (see APPENDIX B, Figure 11A) (Kamiya *et al.*, 2005b). In this closed-loop baroreflex system, CSP equals AP. As noise, Gaussian white-noise was

introduced to the neural and/or peripheral arcs (Figure 1C). As in protocol 2, closed-loop-spontaneous transfer functions were calculated by the simplified method, from AP to SNA as the neural arc (corresponding to the $H_{n\text{-closed-spon}}$) and from SNA to AP as the peripheral arc (corresponding to the $H_{p\text{-closed-spon}}$), while neglecting the noise (see APPENDIX A, Figures 11B, 11C and 11D).

We next examined how the noise modifies the closed-loop-spontaneous transfer functions calculated by the simplified method and renders them different from the open-loop transfer functions by simulating three situations. Because of the closed-loop nature, changes in AP (thus, in CSP) modulate SNA via neural arc transfer function ($H_n$), which in turn change AP via peripheral arc transfer function ($H_p$). In the first simulation, noise is present only in the neural arc (Figure 11B). Regardless of the magnitude of the noise, the closed-loop-spontaneous transfer function for the neural arc is greatly different from the open-loop transfer function $H_n$ (red lines), with markedly increased gain and phase lead versus frequency. In contrast, the closed-loop-spontaneous transfer function for the peripheral arc overlap with the open-loop transfer function $H_n$. In the second simulation, noise is present only in the peripheral arc (Figure 11C). Regardless of the magnitude of the noise, the closed-loop-spontaneous transfer function for the neural arc overlaps with the open-loop transfer function $H_p$, whereas that of the peripheral arc deviates markedly from the open-loop transfer function $H_p$ (red lines). In the third simulation, an incremental noise is present in the neural arc and a small constant noise in the peripheral arc (Figure 11D). Regardless of the magnitude of the noise, the closed-loop-spontaneous transfer function for the neural arc is different from the open-loop transfer function $H_n$ (red lines). However, as the noise in the neural arc increases, the closed-loop-spontaneous transfer function for the peripheral arc approaches the open-loop transfer function $H_p$ and becomes superimposed with respect to gain, phase and coherence functions. These simulations indicate that the presence of noise in the neural and peripheral arcs deteriorates the accuracy of identification of closed-loop-spontaneous transfer function for the neural and peripheral arc, respectively. Importantly, our experimental results (Figure 5) may be consistent with the situation that