noise is predominant in the neural arc rather than in the peripheral arc (Figures 11B and 11D). This might reflect our experimental condition that had little or no noise in the peripheral arc (i.e., perturbation to AP), probably because the body did not move in closed-loop baroreflex and spontaneous resting condition. Therefore, a noise predominantly in the neural arc may be a potential mechanism responsible for our experimental finding that the closed-loop-spontaneous transfer function is inappropriate in identifying the neural arc but partially appropriate in identifying the peripheral arc under resting condition.

Physiological implication (1): Baroreflex is predominantly feedforward rather than feedback in closed-loop-spontaneous condition

While we separate the total arc (CSP input to systemic AP) of the baroreflex system into the neural (CSP input to SNA) and peripheral (SNA input to systemic AP) arc subsystems, the neural and the peripheral arcs are equivalent to the feedback and feedforward arcs, respectively, as reported in earlier studies (Barres *et al.*, 2004; Brychta *et al.*, 2007). Using these terms, our data indicate that baroreflex loop is predominantly feedforward rather than feedback in closed-loop spontaneous resting condition. This may be explained by the block diagram and simulation results shown in Figure 11D. As the noise in the neural arc increases, SNA fluctuates more but becomes less dependent on CSP (baroreceptor pressure input), while the augmented SNA changes strongly control systemic AP via peripheral arc transfer function (H_p) with little interruption by noise in the peripheral arc. As a result, baroreflex control becomes feedforward-like, while the closed-loop-spontaneous transfer function for the peripheral arc approaches the open-loop transfer function H_p. This concept may explain our findings that the closed-loop-spontaneous baroreflex transfer function for the peripheral arc partially matched the open-loop transfer function, whereas that for the neural arc did not (Figure 5). In addition, this concept may also explain other data that in the closed-loop spontaneous condition, spontaneous changes in SNA appeared to precede changes in AP and induce positive AP responses (Figure 9A), and that the closed-loop-spontaneous peripheral arc transfer function was capable of predicting the

time-series AP dynamics from SNA (Figures 9B and 9C).

Physiological implication (2): Potential mechanisms for AP and SNA fluctuations in closed-loop-spontaneous condition

Our experiments of opening and closing the baroreflex loop in individual animals may help to suggest potential mechanisms responsible for AP and SNA fluctuations in closed-loop condition. First, both SNA auto-rhythmicity (pacemaker) and baroreflex mechanisms may contribute to fluctuations of AP and SNA at approximately 0.4 Hz actually observed in closed-loop-spontaneous baroreflex condition (Figure 4B), which is often termed the Mayer wave (Malpas & Burgess, 2000; Barres *et al.*, 2004). It is noteworthy that even in baroreflex open-loop condition, SNA autospectrum shows a small peak at approximately 0.4 Hz (arrowhead, Figure 3B) despite no peak in CSP autospectrum, indicating the existence of SNA auto-rhythmicity at 0.4 Hz, which, which in turn produces systemic AP fluctuation at that frequency (Figure 3B) via peripheral arc transfer function (Figure 3C). This may explain the coherence drop at approximately 0.4 Hz (from CSP to SNA) in open-loop condition (Figure 3C). Furthermore, since closing the baroreflex loop greatly increases the peak of SNA autospectrum (arrowhead, Figure 4B), interaction between the baroreflex neural and peripheral arcs is important for these fluctuations. This may be consistent with the report that bilateral denervation of aortic and carotid sinus baroreceptors eliminated 0.4 Hz oscillations of AP and SNA during sympathoexcitatory stress (Barres *et al.*, 2004). Collectively, SNA auto-rhythmicity (= "origin" activity) and its development and propagation by baroreflex may partly be responsible for the 0.4-Hz fluctuations of AP and SNA in this experimental condition.

Second, although the baroreflex feedback system can theoretically generate oscillations of AP and SNA by itself without other factors (i.e., SNA auto-rhythmicity) in closed-loop condition (Guyton & Harris, 1951; deBoer *et al.*, 1987; Kamiya *et al.*, 2005a), the baroreflex loop theory might not contribute to the 0.4-Hz AP and SNA fluctuations observed in the closed-loop-spontaneous condition, for the following reason. The key point of baroreflex loop theory is that when the gain of total arc baroreflex transfer function is greater

than 1 at the frequency where the phase reaches -2π radians (which we defined as f0), oscillations of AP and SNA will occur at around f0. In our actual data, f0 was approximately 0.13 Hz (Figure 3E), which is consistent with a previous study (Malpas & Burgess, 2000) showing increased 0.1 Hz oscillation of AP during hemorrhage in rabbits. Moreover, the gain at f0 was less than 1 (Figure 3E), indicating no oscillations generated according to the baroreflex loop theory. Therefore, in the closed-loop-spontaneous condition, the baroreflex loop theory would not contribute to the 0.4-Hz fluctuations of AP and SNA.

Lastly, respiratory-mediated fluctuation of AP may contribute to SNA fluctuation via baroreflex mechanisms. In baroreflex open-loop condition (Figure 3B), SNA autospectrum shows no peak whereas systemic AP shows a large peak at the frequency of artificial respiration (approximately 0.57 Hz) (Figure 3B). This indicates that AP fluctuation at that frequency is not mediated by SNA but by mechanical aspects of respiration (i.e., respiratory changes in intrathoracic pressure), consistent with an earlier report (Brychta et al., 2007). Since closing the baroreflex loop produces a peak in the SNA autospectrum at the respiratory frequency (Figure 4B), baroreflex mechanism may partly be responsible for respiratory-mediated SNA fluctuation in this experimental condition.

Physiological implication (3): open-loop baroreflex neural arc transfer function is able to predict closed-loop time-series SNA response to drug-induced AP change

The data discussed so far were obtained using mechanical intervention to change carotid sinus pressure, which arguably are not physiological changes. To validate whether the predictabilities of the open-loop and closed-loop-spontaneous transfer functions apply to more physiological conditions, we tested the neural arc transfer functions using pharmacological pressure intervention (phenylephrine and nitroprusside infusions) under closed-loop condition. First, the SNA predicted by open-loop baroreflex neural arc transfer function ($H_{n\text{-}open}$) in response to the measured changes in CSP (=AP) was roughly similar to the actually measured SNA with respect to both amplitude and timing of neural activity (Figure 10A, third and fourth panel), showing high correlation (r^2 =0.9, Figure 10B). This

result indicates that with regard the neural arc, open-loop transfer function is able to predict time-series SNA output from AP input even during pharmacological pressure interventions. A possible explanation for the good predictability is that since the pharmacological interventions exert a noise to the peripheral and not the neural arc, time-series SNA is almost determined by the pharmacologically induced changes in baroreceptor pressure (= systemic AP) via the neural arc transfer function (= $H_{n\text{-}open}$).

In contrast to the open-loop transfer function, the SNA predicted by closed-loop-spontaneous baroreflex neural arc transfer function ($H_{n\text{-}closed\text{-}spon}$) was different from the measured SNA. The predicted SNA was an oppositely directed neural response: when AP (=CSP) increased, the predicted SNA increased whereas the measured SNA decreased, and vice versa. Therefore, with regard the neural arc, the closed-loop-spontaneous transfer function is not able to predict SNA dynamics from AP. The failure in predicting SNA change may be explained by inappropriate system identification in closed-loop condition. Because of the closed-loop condition, the calculated phase function of $H_{n\text{-}closed\text{-}spon}$ was the inverse of that of $H_{p\text{-}closed\text{-}spon}$. Indeed, the phase led as frequency increased (Figure 5, 11B and 11D) in contrast to the phase lag of open-loop transfer function ($H_{n\text{-}open}$). Furthermore, the calculated phase of $H_{n\text{-}closed\text{-}spon}$ resulted in oppositely directed response of predicted SNA as compared with actually measured SNA, in contrast to the good match of predicted SNA by $H_{n\text{-}open}$. These results indicate that with regard the neural arc, open-loop neural arc transfer function predicts time-series SNA response to changes in AP induced by pharmacological interventions, while closed-loop-spontaneous transfer function cannot predict SNA response.

Although spontaneous baroreflex measures are believed to represent the neural arc function (baroreflex control of SNA), the present study raises potential methodological issues. First, since baroreflex is a closed-loop feedback system, there is theoretical difficulty in identifying system characteristics in closed-loop spontaneous condition. Since a relation calculated from SNA input to AP during their spontaneous fluctuations is the inverse of that calculated from AP input to SNA, the calculation itself cannot determine the causality

between them. Our present data clearly indicate limitation in estimating closed-loop spontaneous transfer function of the neural arc, and that a good estimation requires opening the loop and introducing an intervention to the loop. Furthermore, although the spontaneous baroreflex transfer function obtained in closed-loop condition (Orea *et al.*, 2007; Cooke *et al.*, 2009; Ogoh *et al.*, 2009) has been used as surrogates for the neural (= feedback) arc function of the baroreflex loop, it actually represents the peripheral (= feedforward) arc function since baroreflex loop is predominantly feedforward rather than feedback in closed-loop-spontaneous condition.

Second, a recent study (Hart *et al.*) has reported that spontaneous baroreflex measures (slope of strength of muscle SNA burst over binned or nonbinned AP) did not correlate (r^2= 0.05-0.13) with the "gold standard" modified Oxford analysis (nitroprusside and phenylephrine), whereas spontaneous threshold measure (slope of % occurrence of muscle SNA burst over 1 mmHg binned AP, eliminating strength of SNA burst) correlated with it mildly (r^2= 0.5). Although we cannot directly compare the transfer function analysis in our present study with the spontaneous threshold measure reported by Hart *et al.* (2010) because of methodological differences, our open-loop transfer function of the neural arc was able to predict occurrence and magnitude of time-series SNA with a higher degree of accuracy (r^2= 0.9, Figure 10B) and reproduce the AP-SNA relationship during closed-loop, drug-induced AP changes (Figure 10D).

Limitations

The present study has several limitations. First, we excluded the efferent effect of vagally mediated arterial baroreflex, which could affect the properties of baroreflex control of SNAs. Second, artificial respiration and surgical procedures used in this study could affect baroreflex. Third, anaesthetic agents tend to inhibit efferent SNA and depress the gain of baroreflex control of SNA. Fourth, since the present study was animal research, it is limited to applicable to humans. However, a problem of difficulty in identifying "closed-loop" system in contrast with "open-loop" system is common in animal and human studies. Lastly, we

perfused the carotid sinuses with physiological saline pre-equilibrated with atmospheric. Local hypoxia could have occurred and somewhat affected baroreflex control of SNA. Further research to examine the relevance of the present findings to true physiological conditions is necessary.

Summary

In summary, the open-loop baroreflex transfer functions for the neural and peripheral arcs allowed good prediction of the time-series SNA and AP outputs from baroreceptor pressure and SNA inputs, respectively. In contrast, the closed-loop-spontaneous baroreflex transfer function for the neural arc deviated greatly from the open-loop transfer function, and could not predict the time-series SNA dynamics. However, the closed-loop-spontaneous baroreflex transfer function for the peripheral arc partially matched the open-loop transfer function, with reasonable predictability of the time-series AP dynamics although slightly inferior in accuracy. Furthermore, the predictabilities of open-loop and closed-loop-spontaneous transfer functions of the neural arc 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, whereas SNA responses predicted by closed-loop-spontaneous transfer function deviated greatly and were the inverse of measured responses. Therefore, although the spontaneous baroreflex transfer function obtained by closed-loop analysis has been believed to represent the neural arc function, it is indeed inappropriate for system identification of the neural arc but is partially appropriate for system identification of the peripheral arc under resting condition, compared with open-loop analysis.

APPENDIX A

In a block diagram of the open-loop baroreflex system (Figure 1A), CSP is independent of systemic AP because of vascular isolation of the carotid-sinus regions. In this framework, input-output relations of these arcs are expressed in the frequency domain as:

$$SNA(f) = H_n(f) \cdot CSP(f) + NN(f) \quad \text{--- A1}$$

$$AP(f) = H_p(f) \cdot SNA(f) + PN(f) \quad \text{--- A2}$$

where *CSP(f)*, *SNA(f)* and *AP(f)* are the fast Fourier transforms of CSP, SNA and systemic AP, respectively. $H_n(f)$ and $H_p(f)$ denote the neural arc and the peripheral arc transfer functions, respectively. *NN(f)* and *PN(f)* represent unknown noise in the neural and peripheral arcs, respectively.

In the neural arc, calculating the ensemble averages of cross-powers between the terms of Eq. A1 and $CSP(f)$ yields

$$E[SNA(f) \cdot CSP(f)^*] = H_n(f) \cdot E[CSP(f) \cdot CSP(f)^*] + E[NN(f) \cdot CSP(f)^*] \quad \text{--- A3}$$

where $E[\ ]$ indicates an ensemble average operation. $CSP(f)^*$ denotes the complex conjugate of $CSP(f)$. Because $H_n(f)$ is supposed to be time invariant during the observation period, $H_n(f)$ is outside the ensemble average operation. When *CSP* is a white noise signal, $E[NN(f) \cdot CSP(f)^*]$ diminishes to zero asymptotically because the white noise is statistically independent of any other noise signal. Thus we can estimate $H_n(f)$ by the following equation, which we designate $H_{n\text{-}open}(f)$.

$$H_n(f) = \frac{E[SNA(f) \cdot CSP(f)^*]}{E[CSP(f) \cdot CSP(f)^*]} = H_{n-open}(f) \quad \text{--- A4}$$

Similarly, in the peripheral arc, calculating ensemble averages of cross-powers between terms of Eq. A2 and $SNA(f)$ yields

$$E[AP(f) \cdot SNA(f)^*] = H_p(f) \cdot E[SNA(f) \cdot SNA(f)^*] + E[PN(f) \cdot SNA(f)^*] \quad \text{--- A5}$$

where $SNA(f)^*$ denotes the complex conjugate of $SNA(f)$. Because $H_p(f)$ is supposed to be time invariant during the observation period, $H_p(f)$ is outside the ensemble average

operation. In open-loop condition, since *PN(f)* cannot affect *SNA(f)* and is statistically independent of *SNA(f)* by definition, $E[PN(f)\cdot SNA(f)^*]$ diminishes to zero asymptotically. Thus we can estimate $H_p(f)$ by the following equation, which we designate $H_{p\text{-}open}(f)$.

$$H_p(f) = \frac{E[AP(f)\cdot SNA(f)^*]}{E[SNA(f)\cdot SNA(f)^*]} = H_{p-open}(f) \qquad \text{--- A6}$$

In contrast to the open-loop condition, *CSP* is matched with systemic AP in the closed-loop-spontaneous baroreflex condition (Figure 1B). Thus the input-output relations of the arcs in the frequency domain are expressed as:

$$SNA(f) = H_n(f)\cdot AP(f) + NN(f) \qquad \text{--- A7}$$

$$AP(f) = H_p(f)\cdot SNA(f) + PN(f) \qquad \text{--- A8}$$

In the neural arc, calculating ensemble averages of cross-powers between the terms of Eq. A7 and *AP(f)* yields

$$E[SNA(f)\cdot AP(f)^*] = H_n(f)\cdot E[AP(f)\cdot AP(f)^*] + E[NN(f)\cdot AP(f)^*] \qquad \text{--- A9}$$

$$H_n(f) = \frac{E[SNA(f)\cdot AP(f)^*]}{E[AP(f)\cdot AP(f)^*]} - \frac{E[NN(f)\cdot AP(f)^*]}{E[AP(f)\cdot AP(f)^*]} \qquad \text{--- A10}$$

However, in the baroreflex closed-loop conditions, the unknown noise in *SNA* (*NN*) can affect *AP* through the peripheral arc transfer function (H_p). In other words, *AP(f)* inevitably correlates with *NN(f)*, and $E[NN(f)\cdot AP(f)^*]$ does not diminish to zero. $H_n(f)$ cannot be determined because the unknown noise *NN* is practically impossible to quantify. Therefore in protocol 3, we simplify Eq A10 by neglecting the last term, and define the closed-loop-spontaneous transfer function by the following equation, which we designate $H_{n\text{-}closed\text{-}spon}(f)$.

$$H_n(f) = \frac{E[SNA(f)\cdot AP(f)^*]}{E[AP(f)\cdot AP(f)^*]} = H_{n-closed-spon}(f) \qquad \text{--- A11}$$

However, from Eqs. A4 and 11, it is evident that $H_{n\text{-}closed\text{-}spon}(f)$ should be different from $H_{n\text{-}open}(f)$ when *NN(f)* is large and cannot be neglected.

In the peripheral arc, calculating ensemble averages of cross-powers between the terms of Eq. A8 and *SNA*(f) yields:

$$E[AP(f)\cdot SNA(f)^*] = H_p(f)\cdot E[SNA(f)\cdot SNA(f)^*] + E[PN(f)\cdot SNA(f)^*] \quad \text{--- A12}$$

$$H_p(f) = \frac{E[AP(f)\cdot SNA(f)^*]}{E[SNA(f)\cdot SNA(f)^*]} - \frac{E[PN(f)\cdot SNA(f)^*]}{E[SNA(f)\cdot SNA(f)^*]} \quad \text{--- A13}$$

However, in the baroreflex closed-loop conditions, the unknown noise in *AP* (*PN*) can affect *SNA* through the neural arc transfer function (H_n). In other words, *SNA*(f) inevitably correlates with *PN*(f), and $E[PN(f)\cdot SNA(f)^*]$ does not diminish to zero. $H_p(f)$ cannot be determined because the unknown noise *PN* is practically impossible to quantify. Therefore in protocol 3, we simplify Eq A13 by neglecting the last term and define the closed-loop-spontaneous transfer function by the following equation, which we designate $H_{p\text{-}closed\text{-}spon}(f)$.

$$H_p(f) = \frac{E[AP(f)\cdot SNA(f)^*]}{E[SNA(f)\cdot SNA(f)^*]} = H_{p-closed-spon}(f) \quad \text{--- A14}$$

However, from Eq. A6 and A14, it is evident that $H_{p\text{-}closed\text{-}spon}(f)$ should be different from $H_{p\text{-}open}(f)$ when *PN*(f) is large and cannot be neglected.

APPENDIX B

In rabbits, the transfer function of the baroreflex neural arc (baroreceptor pressure/CSP to SNA) approximates derivative characteristics in the frequency range below 0.8 Hz, and high-cut characteristics of frequencies above 0.8 Hz (Kawada *et al.*, 2002). Therefore, according to our previous study, we model the neural arc transfer function (H_N) using Eq. B1 as follows

$$H_N(f) = -K_N \frac{1+\frac{f}{f_{c1}}j}{\left(1+\frac{f}{f_{c2}}\right)^2}\exp(-2\pi fjL) \qquad \text{--- B1}$$

where f and j represent the frequency (in Hz) and imaginary units, respectively; K_N is static gain (in a.u./mmHg); fc1 and fc2 ($fc_1<fc_2$) are corner frequencies (in Hz) for derivative and high-cut characteristics, respectively; and L is a pure delay (in s), that would represent the sum of delays in synaptic transmission in the baroreflex central pathways and the sympathetic ganglion. The dynamic gain increases in the frequency range from fc_1 to fc_2, and decreases above fc_2. Based on the measured results, we set K_N, fc_1, fc_2 and L at 1, 0.1, 0.8 and 0.2, respectively, in all simulations in Figure 11.

In addition, the transfer function of the baroreflex peripheral arc (SNA to systemic AP) approximates the second-order low-pass filter with a lag time in rabbits (Kawada *et al.*, 2002). Therefore, we model the peripheral arc transfer function (H_P) using Eq. B2 as follows:

$$H_P(f) = \frac{K_P}{1+2\zeta\frac{f}{f_N}j+\left(\frac{f}{f_N}j\right)^2}\exp(-2\pi fjL) \qquad \text{--- B2}$$

where K_P is static gain (in mmHg/a.u.); f_N and ζ indicate a natural frequency (in Hz) and a damping ratio, respectively; and L is a pure delay (in s), that would represent the sum of delays in synaptic transmission in the neuroeffector junction and intracellular signal transduction in the effector organs. Based on the measured results, we set K_P, f_N, ζ and L

at 1, 0.07, 1.4 and 1.0, respectively, in all simulations in Figure 11.

AUTHOR CONTRIBUTIONS

The experiments were performed at the Department of Cardiovascular Dynamics, National Cerebral and Cardiovascular Center Research Institute. AK mainly contributed to: (1) Conception and design, (2) Collection, analysis and interpretation of data and (3) Drafting the article or revising it critically for important intellectual content. Other authors helped him particularly in (3).

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Table 1. Transfer functions of the baroreflex neural arc (from CSP to SNA) in open-loop and closed-loop-spontaneous conditions

	Open-loop TF ($H_{n\text{-}open}$, CSP to SNA)	Closed-loop-spontaneous TF ($H_{n\text{-}closed\text{-}spon}$, CSP[=AP] to SNA)
Gain (a.u./mmHg)		
0.01 Hz	1.2 ± 0.2	1.8 ± 0.1*
0.1 Hz	2.0 ± 0.3	5.3 ± 2.8*
0.3 Hz	2.6 ± 0.3	14.6 ± 5.7*
Phase (rad)		
0.01 Hz	–2.7 ± 0.2	–5.4 ± 0.4*
0.1 Hz	–3.0 ± 0.1	–3.1 ± 0.4
0.3 Hz	–3.7 ± 0.1	–2.6 ± 0.11*
Coherence		
0.01 Hz	0.8 ± 0.1	0.8 ± 0.2
0.1 Hz	0.9 ± 0.1	0.6 ± 0.2*
0.3 Hz	0.9 ± 0.1	0.4 ± 0.3*
Slope, dB/decade		
0.01 Hz to 0.3 Hz	4.7 ± 0.4	12.1 ± 6.1*
Step response		
Initial response (au)	–2.4 ± 0.2	Oscillating response
Steady-state level (au)	–1.2 ± 0.2	

Values are mean ± SD (n =10). *P < 0.05; open-loop vs. closed-loop-spontaneous conditions. TF; transfer function

Table 2. Transfer functions of baroreflex peripheral arc (from SNA to AP) in open-loop and closed-loop-spontaneous conditions

	Open–loop TF ($H_{p\text{-}open}$, SNA to AP)	Closed -loop-spontaneous TF ($H_{p\text{-}closed\text{-}spon}$, SNA to AP)
Gain (mmHg/au)		
0.01 Hz	0.7 ± 0.2	0.6 ± 0.2
0.1 Hz	0.1 ± 0.1	0.1 ± 0.1
0.3 Hz	0.03 ± 0.01	0.03 ± 0.02
Phase (rad)		
0.01 Hz	–0.8 ± 0.2	–0.9 ± 0.2
0.1 Hz	–3.0 ± 0.2	–3.0 ± 0.2
0.3 Hz	–4.2 ± 0.1	–4.0 ± 0.3
Coherence		
0.01 Hz	0.9 ± 0.1	0.8 ± 0.2
0.1 Hz	0.8 ± 0.2	0.6 ± 0.2*
0.3 Hz	0.9 ± 0.1	0.4 ± 0.3*
Step response		
Steady-state level (mmHg)	–0.7 ± 0.2	–0.6 ± 0.2

Values are mean ± SD (n =10). *P < 0.05; open-loop vs. closed-loop-spontaneous conditions. TF; transfer function

Table 3. Predictive power of baroreflex transfer functions

	Open-loop TF ($H_{n\text{-}open}$, CSP to SNA)		Closed-loop-spontaneous TF ($H_{n\text{-}closed\text{-}spon}$, CSP[=AP] to SNA)	
Neural arc	r^2	(Predicted condition)	r^2	(Predicted condition)
	0.8 ± 0.1*	(Open-loop)	0.1 ± 0.1	(Open-loop)
	0.1 ± 0.2	(Closed-loop-spon)	0.06 ± 0.1	(Closed-loop-spon)
	0.9 ± 0.1*	(Closed-loop-drug)	0.7 ± 0.1 ($r<0^a$)	(Closed-loop-drug)
	Open-loop TF ($H_{p\text{-}open}$, SNA to AP)		Closed -loop-spontaneous TF ($H_{p\text{-}closed\text{-}spon}$, SNA to AP)	
Peripheral arc	r^2	(Predicted condition)	r^2	(Predicted condition)
	0.8 ± 0.1*	(Open-loop)	0.7 ± 0.1*	(Open-loop)
	0.7 ± 0.1*	(Closed-loop-spon)	0.7 ± 0.1*	(Closed-loop-spon)

Values are mean ± SD (n =10). *P<0.05; significant correlation between predicted and measured values. [a]Regarding the predictive power of closed-loop-spontaneous TF in neural arc closed-loop-drug condition, r is less than 0 (r=-0.8 ± 0.1). TF; transfer function, Open-loop; baroreflex open-loop condition where CSP was binary random (white-noise) sequence and independent of systemic AP, Closed-loop-spon; baroreflex closed-loop spontaneous condition where CSP equalled systemic AP, Closed-loop-drug; sequential infusions of phenylephrine and nitroprusside in closed-loop condition where CSP equalled systemic AP.

FIGURE LEGENDS

Figure 1. **A**: Theoretical considerations of the coupling of baroreflex neural and peripheral arcs. Although baroreflex is a negative feedback control system that senses AP by baroreceptors and regulates AP, we opened the loop by changing baroreceptor pressure independent of AP. By measuring SNA, we divided the baroreflex system into the neural arc (from baroreceptor pressure input to efferent SNA via central nervous system) and the peripheral arc (from SNA input to AP via cardiovascular organs system). **B**: Block diagram of open-loop baroreflex system. Because of vascular isolation of carotid-sinus regions, CSP is independent of systemic AP. Noise is introduced to the neural and/or peripheral arcs. **C**: Block diagram of closed-loop-spontaneous baroreflex system, where CSP equals AP. Noise is introduced to the neural and/or peripheral arcs. Because of the closed-loop nature, changes in AP (thus, in CSP) control SNA via neural arc transfer function (H_n), which in turn modulate AP via peripheral arc transfer function (H_p). CSP, carotid sinus pressure; SNA, sympathetic nerve activity; AP, arterial pressure; Hn, neural arc transfer function; Hp, peripheral arc transfer function, NN., unknown noise in the neural arc; PN., unknown noise in the peripheral arc.

Figure 2. Experimental design. In system identification studies, open-loop (protocol 1, CSP was perturbed according to a binary random sequence) and closed-loop-spontaneous (protocol 2, CSP was matched with systemic AP) baroreflex transfer functions were identified from experimental data. In predictability studies, the predictive power of the above transfer functions was tested using independent data (protocols 3, 4 and 5). Protocol 3 and 4 were open-loop and closed-loop-spontaneous baroreflex conditions, respectively. Protocol 5 was pharmacological pressure intervention by phenylephrine and nitroprusside infusions in closed-loop condition. TF, transfer function; CSP, carotid sinus pressure; SNA, sympathetic nerve activity; AP, arterial pressure.

Figure 3. **A**: Typical representative data of one rabbit in protocol 2, showing time series of