Supplementary Material

The potential influence of several factors should be assessed before estimating fractal scaling exponent of R-R interval time series. Here, we briefly describe methods to probe the influence of these factors on estimated scaling exponents, and report their impact for R-R interval time series used in our study.

Adequacy of data length

The variation in the estimated fractal scaling exponents can be substantial for short time series. To verify that the length of R-R interval time series used in our study allows reliable estimation of scaling exponent, we investigated the effect of data length on the variation of scaling exponents as described in the Appendix. Theoretically, the variance of the estimated scaling exponent should scale proportionally to $1/N$, where N is the length of the time series. Figure S1 shows that the reduction in the variance of estimated scaling exponents with increasing time series length (N) reaches an asymptote around ~1300 beats at baseline and ~1700 beats during combined blockade, and suggests that a minimum R-R interval time series length of ~20 minutes is adequate for reliable estimation of monofractal scaling exponent.

Adequacy of sampling rate

The error due to the sampling rate can be accounted for by inspecting the variation in the estimated scaling exponents in the presence of noise. In our study, 500 Hz sampling rate precludes resolution of fluctuations less than 2 ms. We ran 10000 Monte-Carlo simulations using two data sets: one collected at baseline and the other during combined autonomic blockade, both with scaling exponents close to the population mean (0.75 and 1.08). (The same approach with different data sets did not change the results.) For each simulation, we added random noise, sampled from a Gaussian distribution with zero mean and 1.9 ms standard deviation, to the original R-R interval time series, re-estimated the scaling exponent of these "jittered" time series, and assessed the variation in estimated scaling exponents due to presence of noise.

This artificially augmented sampling error in R-R intervals resulted in negligible variation in estimated scaling exponent, with a range of approximately 0.005 (SD = 0.0005) for baseline, and 0.015 for the double blockade (SD = 0.0021) (Figure S2). This variation is two orders of magnitude smaller than the variation observed at the population level (see Results). The apparent shift in mean fractal scaling exponent with noise is expected under combined autonomic blockade, given the magnitude of noise relative to the mean R-R interval under this condition. However, the shift in the mean exponent compared to the actual exponent was only 0.003. Therefore, a 500 Hz sampling rate appears to be sufficient for reliable estimation of fractal scaling exponents.

Effect of controlled breathing on estimated scaling exponents

Controlled breathing contains a periodic component of 4 seconds (4 – 10 beats, depending on the condition) that can affect the magnitude of fluctuations in R-R interval within shorter windows, therefore, estimated fractal scaling exponent. To test whether this periodic component in R-R interval time series has any effect on estimated scaling coefficients, (1) we removed breathing frequency component from each of the R-R interval time series using a notch-filter centered at 0.25 Hz with a bandwidth of 0.16 – 0.34 Hz; (2) for each time series, we generated pass-band filtered white noise (using an elliptical filter of the same bandwidth as the notch-filter) with the same mean power as the notch-filtered frequency-band of the original series; and (3) added this colored noise to the notch-filtered R-R interval time series. This procedure effectively removed the breathing frequency component from the R-R interval time series while conserving the overall spectral power.

After this component was removed, 68% of the R-R interval time series still did not conform to the standard model. Therefore, the general lack of conformity of our R-R interval time series to standard fractal model could not be completely attributed to the frequency component induced by controlled-breathing. Despite the increase in the number of time series that conform the standard model, controlled breathing did not have any significant effect on the estimated scaling exponents (ANOVA; $F_{198,1} = 0.37$, $p = 0.54$; Figure S3), regardless of experimental condition or session. Therefore, observed lack of reproducibility and reliability of fractal scaling exponents in our data sets cannot be attributed to the controlled breathing.

Above considerations suggest that a 20 minutes time series sampled at 500 Hz appears to be sufficient for reliable estimation of scaling exponents for young healthy individuals considered in this study, and controlled breathing does not appear to have a significant impact on the fractal scaling exponents estimated using DFA. However, we cannot comment on the relative impact of these factors on fractal scaling exponents in studies that include different populations. Note that the variation in estimated exponents, especially with shorter R-R interval time series, can be relatively large, and whether the variation induced by these factors is acceptable depends on the magnitude of variation relative to that at the population level. Therefore, the effect of these factors should be assessed in all studies that rely on fractal scaling exponents.

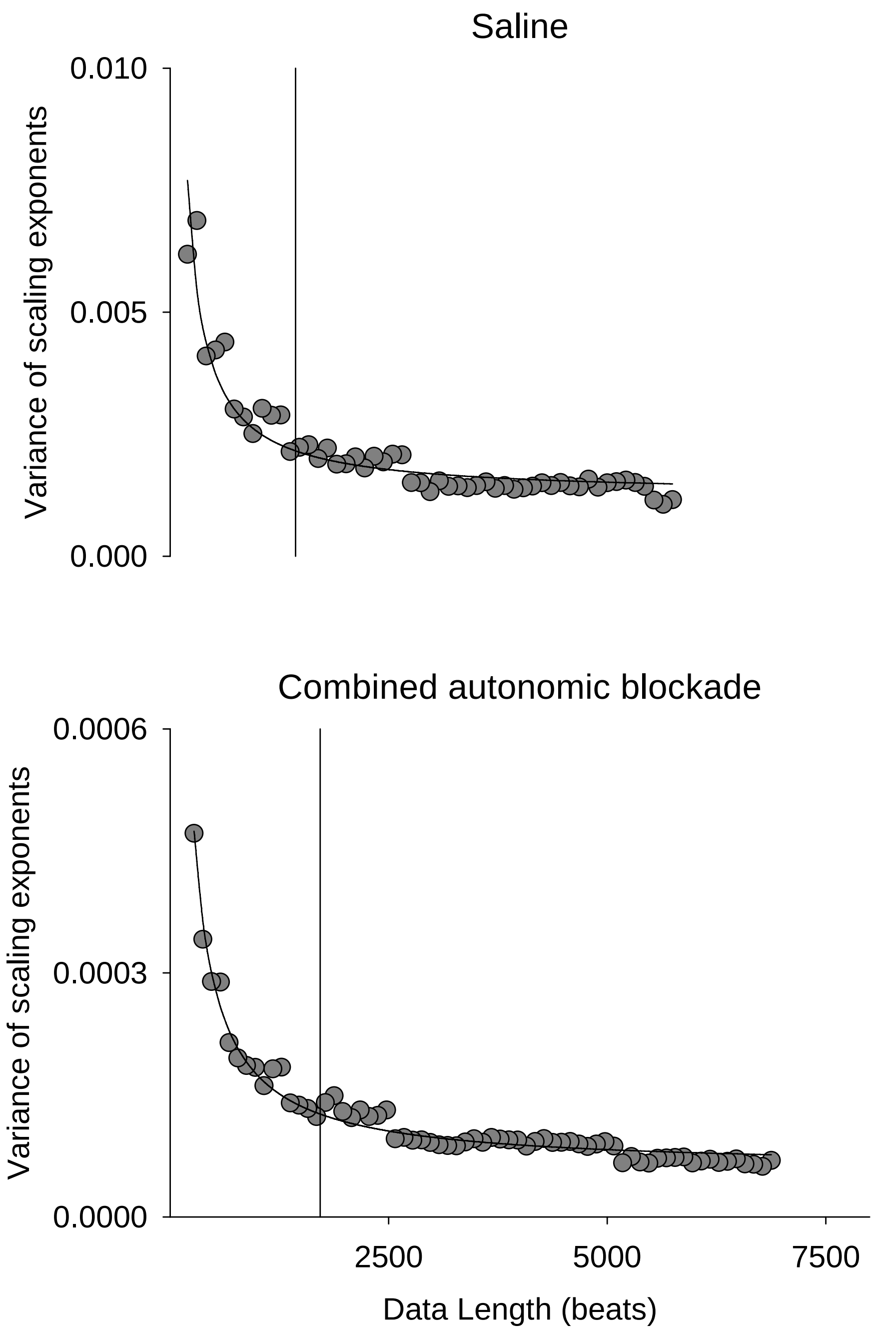
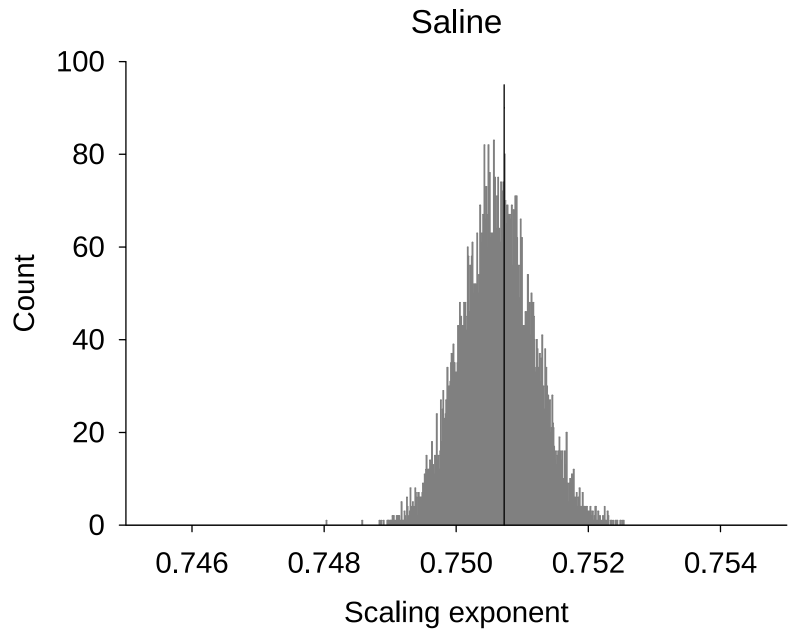


Figure S1: Scaling of the variance of the surrogate data sets for shortest baseline and double blockade R-R interval time series. On each panel, circles show the variance for a given data length (*N*), black curves show the theoretical scaling function proportional to *1/N*, and vertical lines mark the actual length of actual R-R interval time series used to generate surrogate data.



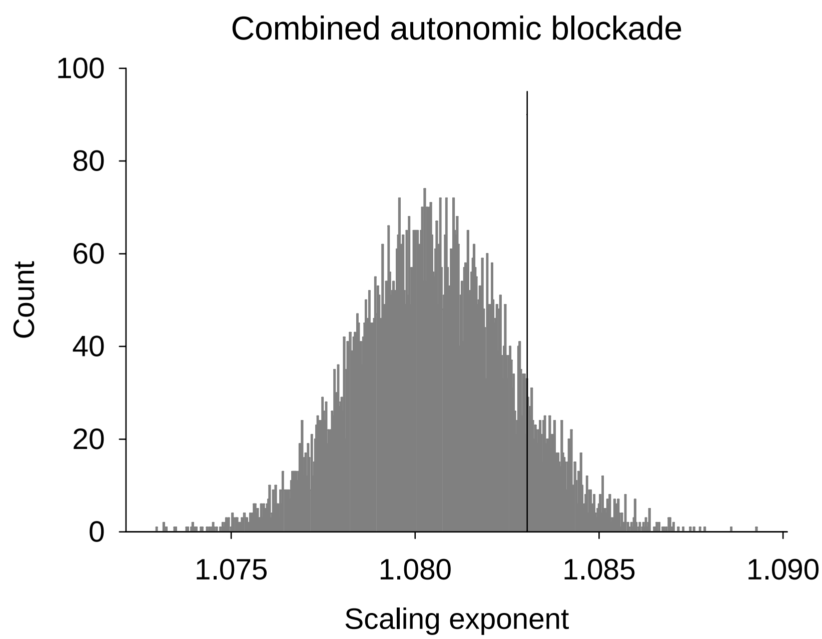


Figure S2: The effect of sampling error on estimated scaling exponents. On both panels, histograms show the distribution of estimated scaling exponents at baseline and during double blockade after “jittering” the R-R interval time series with random noise, and vertical lines mark the actual scaling exponent.

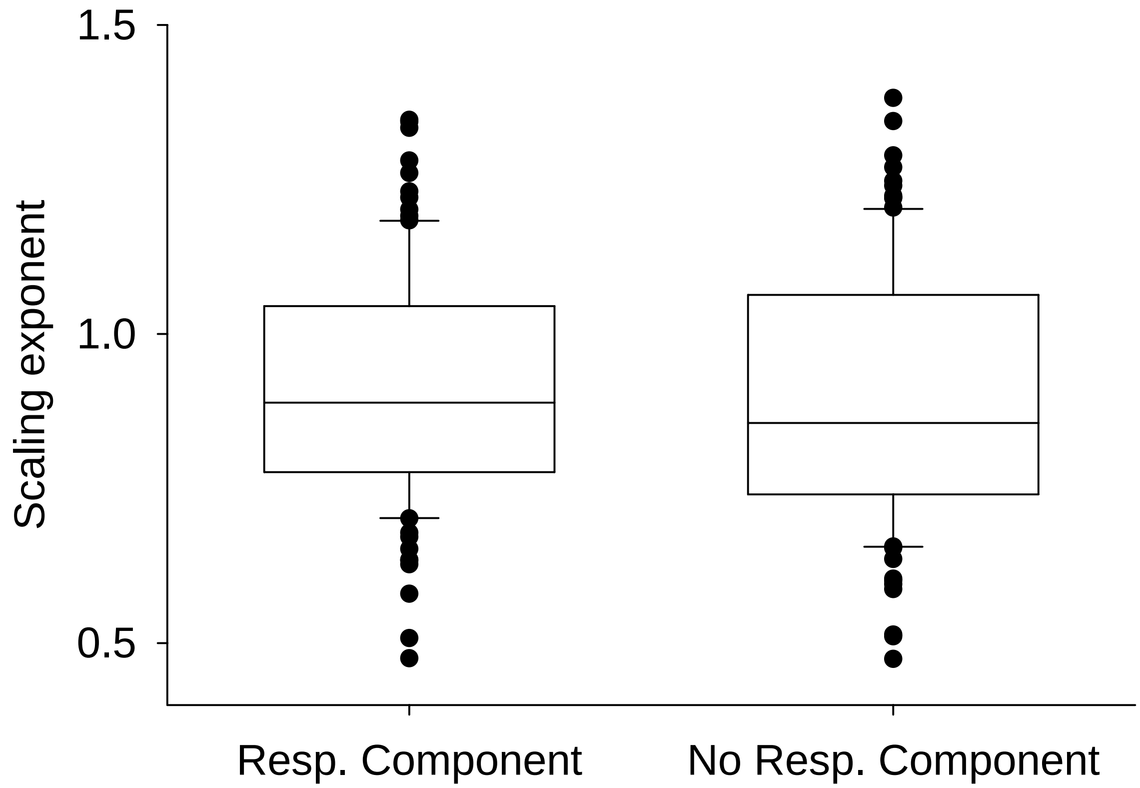


Figure S3: The effect of controlled breathing on estimated scaling exponents. Each line connects the scaling exponents estimated from R-R interval time series to those estimated from the same series after replacing breathing frequency component with noise.