

Webinar Q&A Report

Data Collection & Analysis in Human Autonomic Research: How to Guide to Successful Testing

Questions in this Q&A Report were submitted during the live webinar, [Data Collection & Analysis in Human Autonomic Research: How to Guide Successful Testing](#).

Answers have been provided by Jacqueline Limberg, PhD, Assistant Professor in the Department of Nutrition and Exercise Physiology at the University of Missouri.

1. Can you comment on your experience on the influence of posture on baseline activity and sensitivity in MSNA? What posture is best in your opinion? Are all your subjects studied supine? Do your subjects stay nice and still? If not how do you manage? Do you abort the activity?

We typically conduct our human autonomic studies in the supine posture. Regardless of the posture you choose, consistency is important both between and within individuals. We find the supine posture is the easiest to replicate within and between participants, compared to a semi-recumbent position. It is also the preferred position for participant safety during protocols where we may observe a significant fall in blood pressure (e.g., intravenous nitroprusside). That being said, it's important to acknowledge the supine posture may lower basal MSNA due to elevations in central venous pressure.

More here:

[Postural effects on muscle nerve sympathetic activity in man](#). Burke D, Sundlof G, Wallin G. *J Physiol* 1977 Nov;272(2):399-414. doi: 10.1113/jphysiol.1977.sp012051. PMID: 592197

[Pharmacological assessment of the arterial baroreflex in a young healthy obese male with extremely low baseline muscle sympathetic nerve activity](#). Incognito AV, Samora M, Cartafina RA, Guimaraes GMN, Daher M, Millar PJ, Vianna LC. *Clin Auton Res*. 2018 Dec;28(6):593-595. doi: 10.1007/s10286-018-0559-2. PMID: 30128682

2. What equipment do you use for ETCO2?

ADInstruments has a respiratory gas analyzer that we recently purchased

(<https://www.adinstruments.com/products/gas-analyzer>; Product ML206). We also purchased the CWE GEMINI through ADInstruments, but it costs a little more (<https://www.cwe-inc.com/products/gas-analyzers/gemini-o2-co2-monitor>).

3. What is your opinion on how skin nerve sympathetic activity compares to muscle nerve activity?

Changes in sympathetic outflow are not universal, and certain stressors or maneuvers may increase activity in one vascular bed (muscle), but not in the other (skin).

More on skin sympathetic nerve activity here, including a nice table comparing muscle and skin sympathetic responsiveness to different stimuli:

[Measuring and quantifying skin sympathetic nervous system activity in humans.](#) Greaney JL, Kenney WL. *J Neurophysiol.* 2017 Oct 1;118(4):2181-2193. doi: 10.1152/jn.00283.2017. PMID: 28701539

4. What is your view on combining this setting (HR, NIBP, MSNA) with measurements of blood flow (ultrasound/venous occlusion plethysmography)? Is it possible to do this through LabChart?

You make a very good point. Although muscle sympathetic nerve activity (MSNA) will give you a direct measure of sympathetic activity directed toward the skeletal muscle vasculature, understanding how that MSNA is transduced into a change in vessel diameter, local blood flow, and/or blood pressure is of both clinical and physiological importance. One way to do this is to combine your measures of MSNA with measurements of blood flow (e.g., ultrasound, venous occlusion plethysmography), total peripheral resistance, or blood pressure. This type of “transduction” analysis is possible with LabChart and would allow you the ability to apply additional analyses to your data for a more holistic view.

Here are two examples of this type of analysis:

[Quantifying sympathetic neuro-haemodynamic transduction at rest in humans: insights into sex, ageing and blood pressure control.](#) Briant LJ, Burchell AE, Ratcliffe LE, Charkoudian N, Nightingale AK, Paton JF, Joyner MJ, Hart EC. *J Physiol.* 2016 Sep 1;594(17):4753-68. doi: 10.1113/JP272167. PMID: 27068560

[Influence of age and sex on the pressor response following a spontaneous burst of muscle sympathetic nerve activity.](#) Vianna LC, Hart EC, Fairfax ST, Charkoudian N, Joyner MJ, Fadel PJ. *Am J Physiol Heart Circ Physiol.* 2012 Jun 1;302(11):H2419-27. doi: 10.1152/ajpheart.01105.2011. PMID: 22427525

5. Blood pressure variability is a separate clinically relevant measure. What do you think about short-term blood pressure variability?

This is a very good point that did not make it into the webinar presentation. The sympathetic nervous system plays an important role in low and very low frequency blood pressure variability in

humans. Therefore, low frequency blood pressure variability may be another relatively simple way to examine human autonomic control. Importantly, high frequency blood pressure variability is NOT under sympathetic control and instead is mediated largely by mechanical effects of respiration.

More information in the reference below:

[Mechanism of blood pressure and R-R variability: insights from ganglion blockade in humans](#). Zhang R, Iwasaki K, Zuckerman JH, Behbehani K, Crandall CG, Levine BD. *J Physiol*. 2002 Aug 15;543(Pt 1):337-48. doi: 10.1113/jphysiol.2001.013398. PMID: 12181304

6. Is your definition of mental stress to simply be cognitive work or do you mean stress as in something that may cause some negative emotion on your participant? I ask because from my perspective the Stroop task is quite a simple task. Would a relatively simple task increase autonomic activity to the extent you need?

The idea of a mental stress task in the setting of human autonomic testing would be to introduce the participant to a psychological stressor of some sort which is known to alter autonomic activity (e.g., increase muscle sympathetic nerve activity). The stressor you choose may be dependent on your research question, however during the Webinar Q&A, Professor Vaughan Macefield pointed out that a benefit of the Stroop test is that it can be done on a smart phone/tablet without affecting respiration (the participant doesn't need to "yell" out their response, in contrast to a mental arithmetic test).

More on variability in the autonomic response to different stress tests (mental arithmetic, Stroop): [Rate of rise in diastolic blood pressure influences vascular sympathetic response to mental stress](#). El Sayed K, Macefield VG, Hissen SL, Joyner MJ, Taylor CE. *J Physiol*. 2016 Dec 15;594(24):7465-7482. doi: 10.1113/JP272963. PMID: 27690366

7. What is your opinion on designs that combine stresses? If we do both cold pressor test (CPT) and handgrip exercise/post-exercise ischemia (PEI) at 30% and 45% maximal voluntary contraction (MVC) in one testing session: what is your opinion on order? What about randomizing? Should one go before the other (thinking of the compounding stress)?

It is common practice to combine stressors in your study design. For example, you may hypothesize that metaboreflex activation of the sympathetic nervous system is augmented in a certain population (using post-exercise ischemia), but central processing is similar to control (using cold pressor test). This would require you to complete multiple stressors, with an appropriate wash-out period in between (10-20 minutes) to ensure main outcome variables (HR, BP, MSNA) return to baseline levels.

In studies where multiple stressors are used in a single testing session, it would be ideal to randomize the testing order. However, this is not always realistic. A good example is the use of handgrip exercise at 45% maximal voluntary contraction (MVC). If you are doing a simultaneous

recording of MSNA, it is quite difficult to keep a good MSNA signal at those higher intensities due to movement of the participant. In that instance, you may want to save that test for last to avoid losing your MSNA signal during your other tests.

Example:

[Reflex sympathetic activation during static exercise is severely impaired in patients with myophosphorylase deficiency](#). Fadel PJ, Wang Z, Tuncel M, Watanabe H, Abbas A, Arbique D, Vongpatanasin W, Haley RW, Victor RG, Thomas GD. *J Physiol*. 2003 May 1;548(Pt 3):983-93. doi: 10.1113/jphysiol.2003.039347.

It was also recently shown that the MSNA response to post-exercise ischemia (PEI) has poor-to-moderate within visit reproducibility. So if you plan to do two trials within the same visit, you should keep this information in mind.

More information here:

[Reproducibility of the Neurocardiovascular Responses to Common Laboratory-Based Sympathoexcitatory Stimuli in Young Adults](#). Dillon GA, Lichter ZS, Alexander LM, Vianna LC, Wang J, Fadel PJ, Greaney JL. *J Appl Physiol* (1985). 2020 Sep 17. doi: 10.1152/jappphysiol.00210.2020.. PMID: 32940559

If you have additional questions for Dr. Jacqueline Limberg regarding content from this webinar, or if you wish to receive additional information from [ADInstruments](#) about their instrumentation for human autonomic research, please contact them by phone, or [click here to make a general inquiry](#).

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