Published in final edited form as:

J Child Neurol. 2020 January ; 35(1): 42–48. doi:10.1177/0883073819875915.

Preliminary evidence that resting state heart rate variability predicts reactivity to tactile stimuli in Rett syndrome

Alyssa M. Merbler, MA^{*,a}, Breanne J. Byiers, PhD^{*,a}, John Hoch, PhD, LP^a, Adele C Dimian, PhD^a, Chantel C. Barney, PhD^{a,b}, Timothy J Feyma, MD^b, Arthur A. Beisang, MD^b, Alessandro Bartolomucci, PhD^c, Frank J. Symons, PhD^a

^aDepartment of Educational Psychology, University of Minnesota, Minneapolis, MN, USA

^bGillette Children's Specialty Healthcare, St. Paul, MN, USA

^cDepartment of Integrative Biology and Physiology, University of Minnesota, Minneapolis, MN, USA

Abstract

Patients with Rett syndrome may manifest altered pain perception/experience and are vulnerable to conditions associated with chronic pain. Pain response is difficult to measure, however, because of severe communicative impairment. There is also documented autonomic dysfunction, including decreased heart rate variability. Given the relation between pain and the autonomic nervous system, we tested the feasibility of using resting heart rate variability to predict non-verbal pain/discomfort behavior during a standardized modified quantitative sensory test in Rett syndrome. All stimulus applications resulted in increased behavioral reactivity compared to baseline, with repeated von Frey significantly greater than all other stimuli. Resting heart rate variability predicted behavioral reactivity to repeated von Frey. These preliminary findings provide feasibility evidence for an integrated autonomic-sensory measurement approach and are consistent at a construct level with preclinical evidence in Rett syndrome. Further work is needed to determine how heart rate variability changes during stimulus application.

Keywords

Rett syndrome; MECP2; heart rate variability; quantitative sensory testing

Corresponding Author for Refereeing and Publication: Alyssa Merbler, Merbl004@umn.edu, 250 Education Sciences, 56 East River Rd, Minneapolis, MN 55455, 612-624-5241. **Corresponding Author Post-Publication:** Dr. Breanne Byiers, Byier001@umn.edu, 250 Education Sciences, 56 East River Rd, Minneapolis, MN 55455, 612-626-7892.

^{*}Co-first authors

Author Contributions

AM and BB are co-first authors who contributed equally to the work. AM, BB, AD, and JH collected the data, AM, CB, BB, and FJ contributed to the creation of the behavioral coding system, BB and AM analyzed the cardiac data and behavioral reactivity analyses, respectively, BB performed the statistical modeling, and AB, TF, and AAB contributed to interpretation of the results. BB, AM, and FJ drafted significant portions of the manuscript. All authors critically reviewed the manuscript.

Data Availability

The data are available from the corresponding author, upon reasonable request.

Ethical Approval

This study was approved by the University of Minnesota Human Research Protection Program's Institutional Review Board prior to data collection (IRB number: 1105M99437). All participants' parents/legal guardians provided informed, written consent prior to data collection.

Rett syndrome is a rare and severe developmental neurological syndrome typically caused by loss-of-function mutations in the X-linked methyl-CpG-binding protein 2 (MECP2) gene.

¹ The MeCP2 protein is involved in multiple sensory and nociceptive processes, but specifically how alterations in MeCP2 expression affect pain experience/perception in Rett syndrome is unclear.² Available evidence from preclinical investigations suggests reflexive pain circuitry is intact but that higher-order discriminatory behavior may be impaired (although motor confounds in the behavioral assays may limit their interpretability).³ Decreased sensitivity to pain is among the current supporting diagnostic criteria and reported in previous descriptions of Rett syndrome.^{4,5} Recent work, however, has documented behavioral responses to potentially painful experiences.^{6,7} Although the extant literature points to the likelihood of altered pain experience/perception to at least some degree in Rett syndrome, there has been no work examining modality-specific somatosensory function in patient populations to date. This may be, in part, due to the difficulty of studying somatosensory mechanisms at the patient level, as communicative and motor impairments in Rett syndrome make self-report impossible and imaging difficult.

Autonomic dysfunction is also documented and included in the diagnostic criteria,⁴ including cold hands and feet and breathing and cardiac dysfunction.^{5,8,9} Specific to cardiac function, Rett syndrome samples consistently having lower heart rate variability, on average, correlated with age and clinical severity.^{8,10} Heart rate variability is one metric to quantify autonomic nervous system adaptation to environmental events including endogenous regulatory functions, such as thermal regulation, and acute exogenous events, such as stress or pain.¹¹ Therefore, decreased heart rate variability in Rett syndrome suggests a reduced adaptive ability and thus an increased risk of negative health consequences.¹²

Research investigating the relation of autonomic function and pain has found that resting heart rate variability was related to self-reported pain experiences. Appelhans and Luecken¹³ found that the low frequency component of resting heart rate variability predicted “noticeable” and “moderate” pain thresholds to heat and was correlated with ratings of “unpleasantness” in young, healthy adults.¹³ The authors suggest that their results point to pain experiences, including an affective component, being regulated, in part, by the autonomic nervous system. Examining the specific relation between heart rate variability and sensory experience may help improve our understanding of autonomic function and pain regulation in Rett syndrome. To our knowledge, resting heart rate variability has not been used to predict response to acute tactile or nociceptive stimuli in Rett syndrome. To test whether this was feasible, we modified a quantitative sensory test and examined whether resting heart rate variability predicted pain-relevant, objectively coded behavioral responses (e.g., facial expression) during sensory testing. Because patients were non-verbal, the key modification was based on eliminating individual sensory/pain thresholds as would be done in conventional quantitative sensory testing, but, rather, using constant calibrated tactile sensory stimuli such that the same intensities were used across all patients.

Methods

Participants and Procedures

All procedures were approved by the University of Minnesota's Institutional Review Board. A parent/legal guardian for each participant provided informed consent. Participants were recruited at two consecutive International Rett Syndrome Foundation (now [RettSyndrome.org](https://www.RettSyndrome.org)) family meetings. All families with daughters with clinical diagnoses of Rett syndrome who were present at the meeting were invited to participate. Twenty-seven individuals completed the protocol. Heart rate data files from 10 were deemed to be unusable due to equipment failure ($N = 1$), poor data quality due to excessive movement artifact (i.e., more than 20% of data lost due to artifact; $N = 4$), or frequent episodes of breathing apneas resulting in abnormal HR patterns ($N = 5$). Thus, the final sample consisted of 17 individuals with Rett syndrome with sufficient heart rate data for analysis (Table 1). Two participants (12%) had not undergone genetic testing to confirm the clinical diagnosis. All analyses were replicated with these two participants excluded from the sample, with no changes to the results. As Rett syndrome remains primarily a clinical diagnosis,² these participants were retained in the sample.

Modified Quantitative Sensory Test (mQST)—Six stimuli identical to Barney et al. were applied to the back of each hand and top of each foot in a fixed order: light touch (2g von Frey), pin prick (Medipin), cool (Tip Therm), 4lbs of pressure, repeated von Frey (60g von Frey), and heat (50 °C).¹⁴ Hand stereotypy was allowed to continue during the exam. In rare cases in which hand stereotypy blocked access to the back of the hand for stimulus application, hands were briefly separated to gain access to the back of the hand, but participants were allowed to return to stereotypy. Arms/hands were never restrained.

Analyses

Physiology—Participants were fitted with a Polar s810i soft belt (Polar electro Oy, Finland) around their chest at sternum level to measure inter-beat intervals (RR). Cardiac data were imported, cleaned, and analyzed in VivoSense 3.1 (Vivosense, San Diego, USA). All files were inspected visually for data quality. A 5-minute period in which the participant was seated quietly in a chair was selected as the period for analysis. We chose root mean squared of the differences (RMSSD) of RR intervals as the measure of heart rate variability due to breathing abnormalities in Rett syndrome.¹⁵ The average RMSSD for each 5-minute period was exported and log-transformed to account for the skewed nature of the distribution.

Behavioral Reactivity—We used an observational coding system similar to one used previously in Rett syndrome to assess behavioral responses to each stimulus.^{6,14} The coding system included five classes: *vocalizations* (e.g. moan, cry), *upper and lower facial expressions* (e.g. furrow, mouth open), *motor* (e.g. flinch), and *physiological signs* (e.g. gasp). Each behavior class was scored from 0–3. These classes' scores were summed for each stimulus (max score=15) and across all stimuli for a total score reactivity score (max score = 90). Additionally, a 5-second period prior to the application of stimuli was used to evaluate signs of behavioral reactivity in the absence of tactile stimulation, which served as a

baseline/comparison segment. Videos were coded independently by two coders blind to stimuli and study hypotheses and trained to >85% interobserver agreement, then consensus coded to create scores for analyses. Total pre-consensus interobserver agreement averaged 86.2% (agreements/total number of scored segments x 100).

Analyses—All analyses were conducted in R.^{16–18} An initial analysis was conducted to evaluate differences in behavior scores across stimulus type, accounting for individual variability in baseline reactivity within a linear mixed model. We then analyzed the relation between behavioral reactivity scores for each stimulus and resting RMSSD using repeated-measures linear mixed models with stimulus type as the repeated measures variable, behavior score during each stimulus application as the dependent variable, and age (mean centered, in years), ambulation status (yes/no), and log-transformed resting RMSSD as predictors. A saturated model including two-way interactions between stimulus type and age, ambulation status, and RMSSD was calculated. We then reduced this model via backward elimination model selection by comparing conditional Akaike information criterion (cAIC) values between models.^{19,20} For two-way interactions including stimulus type, the baseline period served as the reference value. Reported p-values are corrected for multiple comparisons using the Benjamini-Hochberg adjustment.

Results

Behavior scores predicted by the stimulus-only linear mixed model are presented in Figure 1. Scores were highest during the repeated von Frey application, followed by light touch, cool, heat, pressure, and pin prick. As anticipated, the lowest scores occurred during baseline. Post-hoc comparisons between stimuli (see Table 2) showed that behavior scores for all stimuli were significantly higher than baseline, with pin prick showing the smallest effect, and the repeated von Frey resulted in behavior scores that were higher than all other stimuli. Difference between the other stimuli were not statistically significant.

The statistical model results are presented in Table 3. Resting heart rate variability ranged from 9.4–65.4ms (mean = 29.2). The corrected AIC value for the full model was 560.67 (df = 37.95), compared to 548.37 (df = 25.23) for the reduced model. In the final model, there was an interaction effect between stimulus type and log-transformed RMSSD, such that those with higher resting heart rate variability had higher repeated von Frey (p=0.001) behavioral reactivity scores (Figure 2). There were no significant main effects or interactions including ambulation status or age.

Discussion

Our purpose was to test whether resting heart rate variability would predict sensory responsiveness in Rett syndrome. From a feasibility perspective, the mQST was successfully implemented with all patients. To our knowledge, this is the first adaptation of quantitative sensory testing in this population. Heart rate variability was successfully collected on all but one participant, but there was 35% data loss due to movement or severe breathing dysfunction.

Given the sample size and study design, we are limited to statements that are sample-specific. Pain- and discomfort-related behavioral reactivity was higher than baseline for all stimuli, demonstrating the perception and response to peripheral sensory information. These results align more broadly with recent work showing evidence of non-verbal pain expression in Rett syndrome.^{6,21} Reactivity to repeated von Frey was the highest of all stimuli. Notably, reactivity scores during the heat and pin prick stimuli, both of which are potentially noxious, were not significantly different than scores during cool and light touch, both of which are reported to be perceived as innocuous by typically-developing adults. Although quantitative sensory testing does not localize pathology along the somatosensory axis, our preliminary observations specific to small unmyelinated fibers indicate possible peripheral involvement based on epidermal nerve density values consistent with the preclinical model.^{22,23}

The lack of response to heat stimulus, along with repeated mechanical stimulation being the highest score, were similar to those in preclinical experimental models at the sensory modality (mechanical, heat) construct level, though we did not see the same patterns for cool and pressure.^{22,24} There are important procedural differences in specificity and precision, though. The clinical von Frey application was not likely delivering the same ‘sensory experience’ as the experimental pre-clinical von Frey applications, which were delivered to a force needed to elicit hind-paw withdrawal. The specific difference is that the pre-clinical approach was designed around threshold (apply stimulus until a withdrawal response – interpretation = ‘nociceptive threshold’) whereas the clinical approach was not threshold, but time/event based (i.e. score behavioral reactivity time-locked to the standardized stimulus application). The similarities are worth pursuing, as it could create a useful bench-translational bridge to advance scientific understanding of sensory/nociceptive function in Rett syndrome.

In terms of autonomic response, resting heart rate variability predicted behavioral reactivity to mechanical stimulation (repeated Von Frey as an indirect, albeit incomplete test, of temporal summation). Reactivity to the other tested sensory modalities was not predicted. This could be due to repeated von Frey have a longer application time than other stimuli and thus a long enough time to register a change in cardiac signal. Taken together, although preliminary, the two approaches to autonomic and sensory assessment, when combined, may help overcome the issue of absent self-report in Rett syndrome without relying on proxy-report in its place, which is a common practice in this and other populations with limited verbal abilities.

Limitations and Future Directions

There are several limitations to this study. First, this was a small convenience sample from Rett syndrome family conferences. Future work should aim for a more representative sample of girls and women with Rett syndrome, as girls and women able to attend conferences may differ from the general Rett syndrome population. Second, pin prick had the lowest behavioral reactivity score, but also had the shortest application. This, coupled with the possibility of delayed motor responding in Rett syndrome,^{5,25} should cause future work in this area to consider increasing the application time and/or the response period after the application to make it more comparable to other stimuli in this mQST procedure. Doing so

would help better determine if the lack of reactivity to pin prick is due to the short response window or alterations in sensory/nociceptive processes. Third, we only examined resting heart rate variability, but future work should analyze how heart rate variability changes during each stimulus application, as this has not yet been explored in Rett syndrome. It has been well established that resting heart rate variability is low in Rett syndrome,⁸ but it is unknown how heart rate variability changes in response to acute environmental challenges. Finally, acquiring useable cardiac data was not successful for all participants recruited. Improvements in equipment and protocols (e.g. electrocardiography leads, movement-resistant electrodes, and the addition respiration measurement) in future studies may help minimize the amount of data loss, increasing the utility of this measure in Rett syndrome.

Our findings represent the possibility of leveraging a known biomarker (heart rate variability) and a novel approach for investigating somatosensory function for use to increase our scientific understanding of phenotypic characteristics in Rett syndrome. As the number of trials in Rett syndrome continues to increase, so too does the need for sensitive, meaningful outcome measures.²⁶ It is unknown, though, whether the measures presented here would be effective biomarkers in clinical trials. Additional work is needed to establish psychometric properties of the sensory measurement approach in relation to cardiac readout more completely. The Food and Drug Administration guidelines for industry clinical trials in rare diseases recommend efficacy outcomes that are tolerable for the participant and staff to acquire, resistant to bias, able to detect change (particularly small change), and meaningfully related to function and/or symptoms.²⁷ Resting heart rate variability has been used as an outcome measure in Rett syndrome, with results showing increased resting heart rate variability post-acetyl-L-carnitine treatment.²⁸ In addition to the possible utility as outcome measures, it is possible that these measures may serve as predictive or diagnostic biomarkers,²⁹ as both heart rate variability and traditional quantitative sensory testing have been used to predict health outcomes in other populations.^{11,30} The work reported on here lays the groundwork for further investigation into the utility, sensitivity, and relevance of a pain/heart rate variability protocol in Rett syndrome.

Acknowledgements

We would like to thank all the participants and their families, as well as [RettSyndrome.org](https://rettsyndrome.org), without whom this work would not be possible. Thanks also to Ashley Nelson, Christopher Lindgren, Mia Koski, and Michael Gleaves for their technical assistance coding the behavioral data for this project.

We would also like to thank the National Institute of Child Health and Human Development, [RettSyndrome.org](https://rettsyndrome.org) (formerly the International Rett Syndrome Foundation), and the Mayday Fund for their support.

Funding

This work was supported, in part, by the National Institute of Child Health and Human Development grant No. 44763, [RettSyndrome.org](https://rettsyndrome.org) (formerly the International Rett Syndrome Foundation), and the Mayday Fund. No funder had involvement in the study design, collection, analysis, interpretation of the data, or preparation or submission of this manuscript.

Declarations of Interest: Drs. Byiers, Hoch, Dimian, and Symons declare funding from the National Institute of Child Health and Human Development. Ms. Merbler and Drs. Byiers and Dimian declare funding from [RettSyndrome.org](https://rettsyndrome.org) (formerly International Rett Syndrome Foundation). Dr. Barney declares funding from the Mayday Fund. The other authors declare no conflicts of interest related to this study.

References

1. Amir RE, Van den Veyver IB, Wan M, Tran CQ, Francke U, Zoghbi HY. Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat Genet.* 1999 10;23(2):185–8. doi: 10.1038/13810. [PubMed: 10508514]
2. Martin P Pain in Rett syndrome: peculiarities in pain processing and expression, liability to pain causing disorders and diseases, and specific aspects of pain assessment. *Adv Autism.* 2017; 3(3):163–82. doi: 10.1108/AIA-02-2017-0003.
3. Samaco RC, Fryer JD, Ren J, Fyffe S, Chao HT, Sun Y, et al. A partial loss of function allele of methyl-CpG-binding protein 2 predicts a human neurodevelopmental syndrome. *Hum Mol Genet* 2008;17(12):1718–27. doi: 10.1093/hmg/ddn062. [PubMed: 18321864]
4. Neul JL, Kaufmann WE, Glaze DG, Christodoulou J, Clarke AJ, Bahi-Buisson N et al. Rett syndrome: revised diagnostic criteria and nomenclature. *Ann Neurol.* 2010 12;68(6):944–50. doi: 10.1002/ana.22124. [PubMed: 21154482]
5. Hagberg B Clinical manifestations and stages of Rett syndrome. *Ment Retard Dev Disabil Res Rev.* 2002;8(2):61–5. [PubMed: 12112728]
6. Barney CC, Feyma T, Beisang A, Symons FJ. Pain experience and expression in Rett syndrome: subjective and objective measurement approaches. *J Dev Phys Disabil.* 2015 8 1;27(4):417–29. doi: 10.1007/s10882-015-9427-3. [PubMed: 26425056]
7. O’Leary HM, Marschik PB, Khwaja OS, Ho E, Barnes KV. Detecting autonomic response to pain in Rett syndrome. *Dev Neurorehabil.* 2017 2;20(2):108–14. doi: 10.3109/17518423.2015.1087437. [PubMed: 26457613]
8. Acampa M, Guideri F. Cardiac disease and Rett syndrome. *Arch Dis Child.* 2006 5;91(5):440–3. doi: 10.1136/adc.2005.090290. [PubMed: 16632674]
9. Symons FJ, Byiers B, Hoch J, et al. Infrared Thermal Analysis and Individual Differences in Skin Temperature Asymmetry in Rett Syndrome. *Pediatr Neurol.* 2015;53(2):169–172. doi:10.1016/j.pediatrneurol.2015.03.018. [PubMed: 26003587]
10. Guideri F, Acampa M, Di Perri T, Zappella M, Hayek Y. Progressive cardiac dysautonomia observed in patients affected by classic Rett syndrome and not in the preserved speech variant. *J Child Neurol.* 2001 5;16(5):370–3. doi: 10.1177/088307380101600512. [PubMed: 11392524]
11. Kleiger RE, Stein PK, Bigger JT Jr. Heart rate variability: measurement and clinical utility. *Ann Noninvasive Electrocardiol.* 2005 1;10(1):88–101. doi: 10.1111/j.1542-474X.2005.10101.x
12. Thayer JF, Yamamoto SS, Brosschot JF. The relationship of autonomic imbalance, heart rate variability and cardiovascular disease risk factors. *Int J Cardiol.* 2010 5 28;141(2):122–31. doi: 10.1016/j.ijcard.2009.09.543. [PubMed: 19910061]
13. Appelhans BM, Luecken LJ. Heart rate variability and pain: associations of two interrelated homeostatic processes. *Biol Psychol.* 2008;77(2):174–182. doi:10.1016/j.biopsycho.2007.10.004. [PubMed: 18023960]
14. Barney CC, Tervo R, Wilcox GL, Symons FJ. A case-controlled investigation of tactile reactivity in young children with and without global developmental delay. *Am J Intellect Dev Disabil.* 2017 9;122(5):409–21. doi: 10.1352/1944-7558-122.5.409. [PubMed: 28846038]
15. Laborde S, Mosley E, Thayer JF. Heart rate variability and cardiac vagal tone in psychophysiological research- recommendations for experiment planning, data analysis, and data reporting. *Front Psychol.* 2017 2 20;8:213. doi: 10.3389/fpsyg.2017.00213. [PubMed: 28265249]
16. Säfken B, Rügamer D, Kneib T, Greven S cAIC4: conditional Akaike information criterion for ‘lme4’. R package version 0.6. <https://cran.r-project.org/package=cAIC4>.
17. Pinheiro J, Bates D, DebRoy S. nlme: linear and nonlinear mixed effects models. R package version 3.1–137. <https://cran.r-project.org/package=nlme>.
18. Luo D, Ganesh S, Koolgaard J. predictmeans: calculate predicted means for linear models. R package version 1.0.1. <https://cran.r-project.org/package=predictmeans>.
19. Cheng J, Edwards LJ, Maldonado-Molina MM, Komro KA, Muller KE. Real longitudinal data analysis for real people: building a good enough mixed model. *Stat Med.* 2010 2 20;29(4):504–20. doi: 10.1002/sim.3775. [PubMed: 20013937]

20. Liang H, Wu H, Zou G. A note on conditional AIC for linear mixed-effects models. *Biometrika*. 2008;95(3):773–8. [PubMed: 19122890]
21. Symons FJ, Byiers BJ, Tervo R, Beisang A. (2013). Parent reported pain in rett syndrome. *Clin J Pain*. 2013 8; 29(8): 744–746. doi: 10.1097/AJP.0b013e318274b6bd [PubMed: 23835769]
22. Bhattacharjee A, Mu Y, Winter MK, Knapp JF, Eggimann LA, Gunewardena SS et al. (2017). Neuronal cytoskeletal gene dysregulation and mechanical hypersensitivity in a rat model of Rett syndrome. *Proc Natl Acad Sci U S A*. 2017 8 15;114(33):E6952–E6961. doi: 10.1073/pnas.1618210114. [PubMed: 28760966]
23. Symons FJ, Barney CC, Byiers BJ, McAdams BD, Foster SXYL, Feyma TJ et al. A clinical case-controlled comparison of epidermal innervation density in Rett syndrome. *Brain Behav*. 2019; e01285. doi: 10.1002/brb3.1285.
24. Chen Y, Yu J, Niu Y, Qin D, Liu H, Li G et al. Modeling Rett syndrome using TALEN-edited MECP2 mutant cynomolgus monkeys. *Cell*. 2017 5 18;169(5):945–55. doi: 10.1016/j.cell.2017.04.035. [PubMed: 28525759]
25. Segawa M Early motor disturbances in Rett syndrome and its pathophysiological importance. *Brain Dev*. 2005 11;27 Suppl 1:S54–S8. doi: 10.1016/j.braindev.2004.11.010. [PubMed: 16182486]
26. International Rett Syndrome Foundation (2010). IRSF Translational Research Meeting New York, NY: International Rett Syndrome Foundation, pp.5–7.
27. Food and Drug Administration. Rare Diseases: common issues in drug development- guidance for industry. <https://www.fda.gov/ucm/groups/fdagov-public/@fdagov-drugs-gen/documents/document/ucm629579.pdf>. Updated January 2019 Accessed November 2018.
28. Guideri F, Acampa M, Hayek Y, Zappella M. Effects of acetyl-L-carnitine on cardiac dysautonomia in Rett syndrome: prevention of sudden death? *Pediatr Cardiol*. 2005 Sep-Oct;26(5):574–7. [PubMed: 16235010]
29. FDA-NIH Biomarker Working Group. BEST (Biomarkers, EndpointS, and other Tools) Resource [Internet]. Silver Spring (MD): Food and Drug Administration (US); 2016. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK326791/Co-published> by National Institutes of Health (US), Bethesda (MD).
30. Treed RD. The role of quantitative sensory testing on the prediction of chronic pain. *PAIN*. 2019;160:S66–9. [PubMed: 31008852]

Figure 1.

Behavioral reactivity by stimulus. Each point represents one participant's score. Reactivity scores for each stimulus were significantly higher than baseline.

Figure 2.

Behavioral reactivity within each stimulus by resting RMSSD. There was a significant interaction for stimulus reactivity and resting RMSSD for repeated von Frey ($p < 0.001$).

Table 1.

Participant Characteristics.

ID	Mutation	Age	Ambulatory	Total behavior score	Resting RMSSD	Percent HRV ^a data cleaned
1	Check	2	Y	40	27	17.7
2	R306C	4	Y	30	65.4	4.7
3	Other point	7	Y	49	21.9	2.7
4	R306C	10	Y	37	21.2	0
5	Other point	11	Y	41	58.8	8
6	R255×	11	N	35	49	5
7	R168×	13	N	43	27.5	3
8	R306C	15	Y	28	44	18.3
9	R255×	15	N	13	13.2	0
10	Deletion	21	Y	20	16.2	0.3
11	T158M	21	N	5	9.4	0
12	R255×	28	Y	32	25.9	2.7
13	Deletion	28	N	6	11.2	17.7
14	Unknown	33	Y	23	26.4	19
15	R294×	34	N	17	24.3	0
16	R168×	35	N	17	26	7.7
17	Unknown	38	Y	35	25	12.3

^aHeart rate variability

Table 2.

Minimum, maximum, and predicted behavior scores by stimulus, and *p* values of inter-stimulus comparisons for behavior scores.

	0	1	2	3	4	5	Min	Max	Predicted value
0. Baseline	-						0	4	1.06
1. Light touch	<0.001	-					0	10	4.47
2. Pin prick	0.053	0.070	-				0	8	2.82
3. Cool	<0.001	0.746	0.145	-			0	9	4.18
4. Pressure	0.004	0.374	0.440	0.535	-		0	9	3.59
5. Repeated von Frey	<0.001	<0.001	<0.001	<0.001	<0.001	-	0	15	8.82
6. Heat	0.002	0.512	0.313	0.725	0.767	<0.001	0	9	3.82

Note: All stimuli have a maximum possible score of 15

Table 3.

Full and Reduced Model Results.

	Sum of Squares	Mean Square	df	F value	p value
Full model terms					
Stimulus	21.24	3.54	6/78	0.76	0.604
Ambulation	9.66	9.66	1/13	2.07	0.174
Age	1.36	1.36	1/13	0.29	0.599
(log) RMSSD	32.20	32.20	1/13	6.91	0.021
Stimulus by (log)RMSSD	47.32	7.89	6/78	1.67	0.134
Stimulus by Age	28.45	4.74	6/78	1.02	0.421
Stimulus by Ambulation	24.67	4.11	6/78	0.88	0.512
Reduced model terms					
Stimulus	34.88	5.81	6/90	1.24	0.292
(log)RMSSD	88.91	88.91	1/15	19.00	<0.001
Stimulus by (log)RMSSD	88.21	14.70	6/90	3.14	0.008
Stimulus by (log) RMSSD post-hoc <i>t</i> -tests					
	Estimate	Standard Error	df	<i>t</i> value	p value
Light Touch by (log)RMSSD	1.07	1.41	90	0.77	0.446
PinPrick by (log)RMSSD	1.75	1.41	90	1.24	0.218
Cool by (log)RMSSD	1.47	1.41	90	1.04	0.299
Pressure by (log)RMSSD	2.04	1.41	90	1.45	0.151
Repeated Von Frey by (log)RMSSD	5.63	1.41	90	4.01	<0.001
Heat by (log)RMSSD	1.46	1.41	90	1.04	0.302