

EFFECTS OF EQUINE AROMATHERAPY ON HEART-RATE VARIABILITY AND  
SALIVARY CORTISOL By  
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With Honors in  
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Approved by:

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## Abstract:

The objective of this project is to determine whether equine aromatherapy can optimize heart-rate variability parameters and decrease salivary cortisol levels. The protocol follows a crossover design with a treatment of humidified essential oil (*Lavandula augustifolia* and *Chamaemelum nobile*) and control of humidified air; HRV measurements for a sample size of 8 dressage horses in each study were collected by placing the respective Polar Equine monitors around the horse's abdomen and recording for 7 minutes each at baseline, during treatment, immediately post-treatment, and 0.5 hour after each treatment. During the chamomile study, salivary cortisol levels were obtained by holding saliva swabs in the horse's mouth for 60-90 sec. Statistically significant increases relative to baseline was discovered with RMSSD during lavender treatment and a statistically significant decrease for VLF during post-treatment in the lavender study; in the chamomile study, there was statistically significant increase in SDNN during post-treatment ( $p < 0.05$ ).

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## Introduction

### **Application:**

Aromatherapy has long been used as a method of stress reduction and present literature demonstrates that humans can reach greater autonomic coherence and balance with aromatherapy used in conjunction with biofeedback (Lee Y-L et al, 2011). Limited research has been performed, however, on the use of equine aromatherapy to optimize heart-rate variability. There is currently only one published paper reporting the effects of aromatherapy exposure on horses (Ferguson et al, 2013), which measured heart rate, which as a parameter alone is not as sensitive, nor does it provide as much information, as HRV. Additionally, the study imposed acute stress on the horses and restrained them in cross-ties, which does not provide accurate data regarding the horse's natural response to the aromatherapy exposure. The aim of this study is to investigate whether aromatherapy can be used to reduce stress levels in horses by improving sympathovagal balance. If we discover that aromatherapy optimizes HRV parameters and promotes greater autonomic control, not only does this provide clinical relevance for improved states in horses (as well as humans) but also offers possible calming techniques in transportation (via airplane and trailer), or may alleviate anxiety/difficult behaviors during clipping or shoeing, as well as improving overall behavior to make the riding experience more pleasant and safe.

### **Context:**

Olfaction stimuli feed directly into the amygdala, a bilateral limbic structure which is highly involved in the stress response and whose functioning is critical in autonomic regulation as well as control of emotions and thought processes. Dysregulation of the amygdala often provokes pathological states. An overactive amygdala, for example, has been shown to confer anxiety

disorders such as PTSD, while an underactive amygdala is found in Parkinson's disease. Both hyper and hypo-activation of the amygdala is associated with low psychological stability and low heart-rate variability (HRV) parameters (Baldwin, Lecture 9).

HRV describes the variation in the inter-beat interval in heart rate measurements, and is essentially an assessor of sympathovagal balance (the close communication between sympathetic and parasympathetic branches of the autonomic nervous system). The benefits of achieving optimal, high levels of heart-rate variability are manifold: high HRV is associated with greater baroreceptor sensitivity (which allows for greater control of blood pressure) as well as overall improved cardiac function, immune function, and brain function (Baldwin, Lecture 5).

**Objective:**

Because the nerve pathways from the nasal sinuses feed directly into the amygdala, there is heightened sensitivity to minute exposure to smells; the sense of smell can induce powerful influences on mental and physical states (in PTSD, olfactory stimuli often trigger episodes of intrusive traumatic recall). It is therefore clinically relevant to examine the utility of aromatherapy in achieving increased HRV.

## Materials and Methods:

### Animal Subjects Approval:

This study was approved by the University of Arizona's Institutional Animal Care and Use Committee.

### Funding:

This work was supported by the University of Arizona's Honors College Alumni Legacy Grant.

### Participants:

9 dressage horses stabled at Willow Woods Farm (3312 N Riverbend Circle East, Tucson, AZ 85750) were selected for this study. The horses varied in age and breed; the profiles for each horse are listed below in Table 1.1.

| Name   | Age (yrs) | Gender | Breed                      |
|--------|-----------|--------|----------------------------|
| Pluto  | 29.5      | Male   | Lipizzaner                 |
| Moon   | 15        | Male   | American Saddlebred        |
| Dewie  | 18        | Male   | Thoroughbred               |
| Bianca | 18        | Female | Azteca                     |
| Remi   | 17        | Male   | Appaloosa / American Paint |
| Anna   | 8         | Female | Oldenburg                  |
| Flash  | 14        | Male   | Morgan                     |
| Daphne | 7         | Female | Dutch Warmblood            |
| Major  | 16        | Male   | American Quarter           |

**Table 1.1:** Age, gender, and breed profiles for each horse that participated in the lavender study and the chamomile study

### Design:

The experimental protocol follows a crossover design with a treatment of humidified essential oil and a control of humidified air (water). Each horse received both the essential oil intervention and the water control, which allowed for each horse to serve as their own control to

account for possible confounding variables such as personality, breed, and other individual differences between the horses.

This project investigated the effects of two essential oils: lavender (*Lavandula augustifolia*) and chamomile (*Chamaemelum nobile*), and was divided into a lavender study and a chamomile study. Each study consisted of a sample size of eight (N =8) horses; every horse participated in both the lavender and the chamomile study, except for Daphne and Major. Daphne relocated and was unable to participate in the chamomile study; Major was recruited into the chamomile study as her replacement. The sample size was chosen based on a previous equine aromatherapy study performed (Ferguson et al, 2013) in which a sample of seven (N = 7) horses was sufficient to yield statistically significant changes in heart rate.

In both studies, drops of either 100% *Lavandula augustifolia* or 100% *Chamaemelum nobile* essential oil treatments (PlantLife, San Clemente, CA) or water control were placed onto the filter pad of an essential oil diffuser (SpaRoom Scientifier); separate diffusers were used for essential oil treatments versus water control. The diffuser was held directly in front of the horse's nose, expelling either humidified lavender or air (Figure 1.1)



**Figure 1.1:** Diffuser expelling either humidified essential oil or water control treatments is held directly in front of the horse's nose for duration of 7 minutes during the treatment period.

## **Experimental Measures:**

### *Heart-Rate Variability Testing Procedure*

HRV measurements were collected using the Polar Equine RS800CX Science Heart Rate Monitor (Polar, Warminster, PA, USA). The monitor consisted of an electrode transmitter which attached to a strap that is placed around the horse's abdomen and a wristwatch receiver which is worn by the investigator collecting data (Figure 1.2). HRV recordings for duration of seven minutes each were taken at baseline, during treatment, immediately post-treatment, and a half-hour after each treatment. Each period was also video-taped for future analysis of behavioral responses.



**Figure 1.2:** Depicts Polar Equine Heart Rate Monitor; the electrode transmitter is attached to a strap and is worn around the horse's abdomen; the wristwatch receiver is worn by the investigator.

### *Heart-Rate Variability Data Analysis*

Extraction of the HRV data was accomplished using Polar ProTrainer Equine Edition software, and interpretation of the data was performed using the Kubios HRV platform, which allowed for the following parameters to be analyzed: heart-rate, SDNN, RMSSD, VLF (%), LF (%), and HF



(%). SigmaStat software was used to perform paired T-tests (and Signed Rank tests when normality failed) to test for statistically significant changes in each parameter from baseline.

*Definitions of Heart-Rate Variability Parameters:*

- **Heart Rate**: number of beats per minute that the heart contracts
- **SDNN**: standard deviation of the intervals between each R peak on an ECG (the inter-beat interval); reflects amplitude of HRV
- **RMSSD**: root mean square of successive differences between the inter-beat intervals; indicator of parasympathetic tone
- **VLF**: very low frequency power,  $<0.003 - 0.04\text{Hz}$ , degree of modulation of sympathetic tone
- **LF**: low frequency power,  $0.04 - 0.15\text{Hz}$ , degree of modulation of sympathetic and parasympathetic tone
- **HF**: high frequency power,  $0.15 - 0.4\text{Hz}$ , degree of modulation of parasympathetic tone

(Baldwin, Lecture 5)

*Salivary Cortisol Testing Procedure:*

The Honors College Alumni Legacy Grant was awarded and allowed for funding to obtain salivary cortisol measures. A pilot study was first performed with two horses, Ely and Major, to determine the time course in salivary cortisol levels after 7 minutes of aromatherapy treatment.

The saliva swabs were sent for analysis to Salimetrics, LLC and the results are reported in Table #). It was determined that salivary cortisol measurements taken after treatment were best sampled 20 minutes after treatment.

During the actual chamomile study, baseline salivary cortisol samples were taken immediately after obtaining baseline HRV recordings and post-treatment samples were taken 20 minutes after the treatment period terminated, as determined from the pilot study.

Saliva samples were obtained using long, cylindrical saliva swabs (SalivaBio Children's Swab) which were held at one end using forceps (Figure 1.3) and were placed under the tongue or between the gum and cheek of the horse's mouth for 60 – 90 seconds until the swab became saturated (Figure 1.4). Swabs were stored in sealed swab storage tubes and frozen at below -20°C. After collecting all the saliva samples with the completion of the chamomile study, the frozen samples were sent over dry ice for analysis to Salimetrics, LLC. *The cortisol samples for the chamomile study are presently still being analyzed and are not included in this paper. Only the cortisol levels collected during the pilot study are currently available.*



**Figure 1.3:** Depicts the long, cylindrical SalivaBio Children's Swab used to collect salivary cortisol samples. One end of the swab is held by the experimenter with forceps.



**Figure 1.4:** Depicts the experimenter holding the saliva swab in the horse's mouth. For safety, one experimenter is assisting with holding the lead rope of the horse.

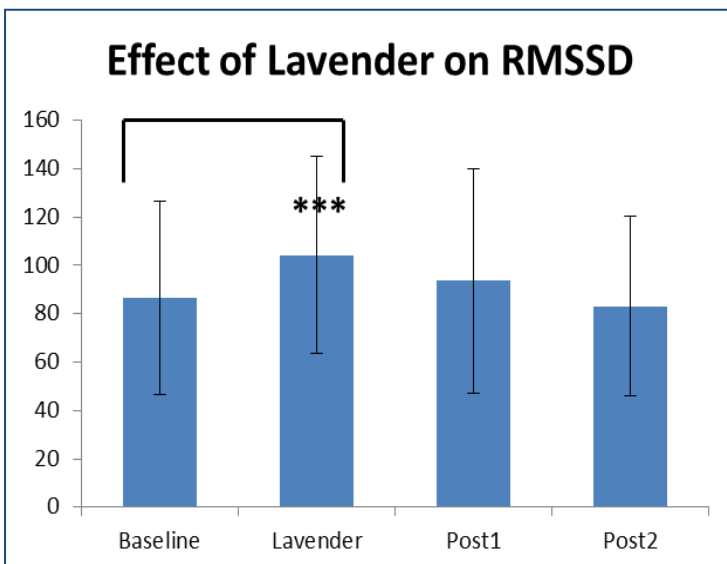
## Results:

### Statistical significance:

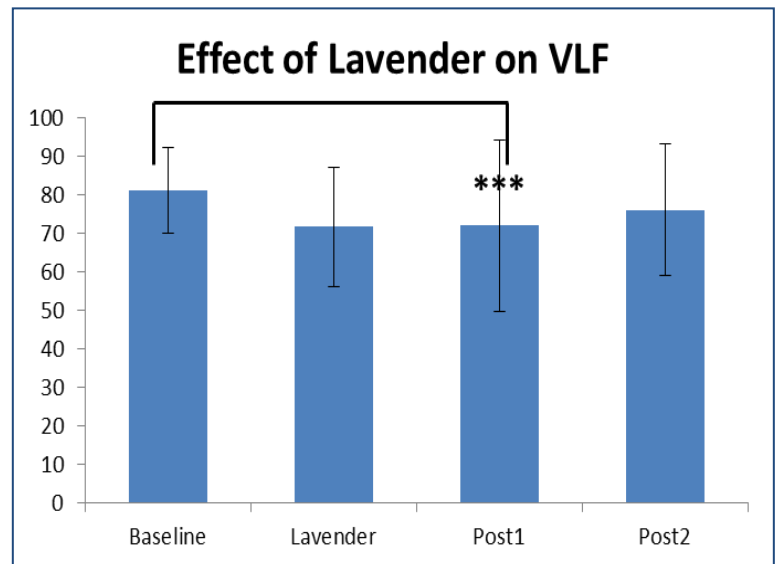
#### *Lavender study*

In the lavender study, there was a statistically significant increase in RMSSD from baseline to lavender treatment (Figure 2.1) from an overall mean of  $86.5 \pm 40.1$  ms to  $104.3 \pm 40.6$  ms ( $p = 0.02$ ). There was a decrease in VLF from both baseline to lavender treatment ( $81.2 \pm 11.0$  ms to  $71.7 \pm 15.4$  ms) and baseline to post-1 ( $81.2 \pm 11.0$  ms to  $72.1 \pm 22.3$  ms), although it was only statistically significant for post-1 ( $p = 0.039$ ) (Figure 2.2). No statistical significance was found compared to baseline for any HRV parameter in the water control treatment. The overall means and standard deviations for each parameter in both the lavender and water control treatments are listed in Table 2.1 and 2.2. Measurements for all the parameters in each individual horse are found in Table 2.3, 2.4, 2.5, and 2.6 in Appendix A.

#### *Graphs of statistically significant changes from baseline*



**Figure 2.1:** Graph depicting means for RMSSD measurements during lavender treatment. Asterisks and bars denote a statistically significant increase in RMSSD from baseline ( $p < 0.05$ )



**Figure 2.2:** Graph depicting means for VLF measurements during lavender treatment. Asterisks and bars denote a statistically significant decrease in VLF (%) from baseline ( $p < 0.05$ )

*Tables of overall means and standard deviations for each HRV parameter*

| Lavender   |                |                |                |                |
|------------|----------------|----------------|----------------|----------------|
|            | Baseline       | Lavender       | Post 1         | Post 2         |
| HR (bpm)   | 36.7 (± 3.9)   | 36.1 (± 3.7)   | 37.2 (± 4.2)   | 40.0 (± 7.3)   |
| SDNN (ms)  | 173.1 (± 74.1) | 170.2 (± 82.9) | 180.9 (± 76.7) | 139.8 (± 46.5) |
| RMSSD (ms) | 86.5 (± 40.1)  | 104.3 (± 40.6) | 93.7 (± 46.4)  | 83.1 (± 37.2)  |
| % VLF      | 81.2 (± 11.0)  | 71.7 (± 15.4)  | 72.1 (± 22.3)  | 76.1 (± 17.1)  |
| % LF       | 12.4 (± 6.1)   | 17.7 (± 8.4)   | 15.5 (± 9.1)   | 12.6 (± 6.9)   |
| % HF       | 6.4 (± 7.5)    | 10.6 (± 12.4)  | 12.4 (± 20.7)  | 11.2 (± 14.7)  |

**Table 2.1:** Means and standard deviations of HRV parameters measured during lavender treatment. Statistical significance from baseline was found in RMSSD during Lavender treatment and in VLF (%) during post-1.

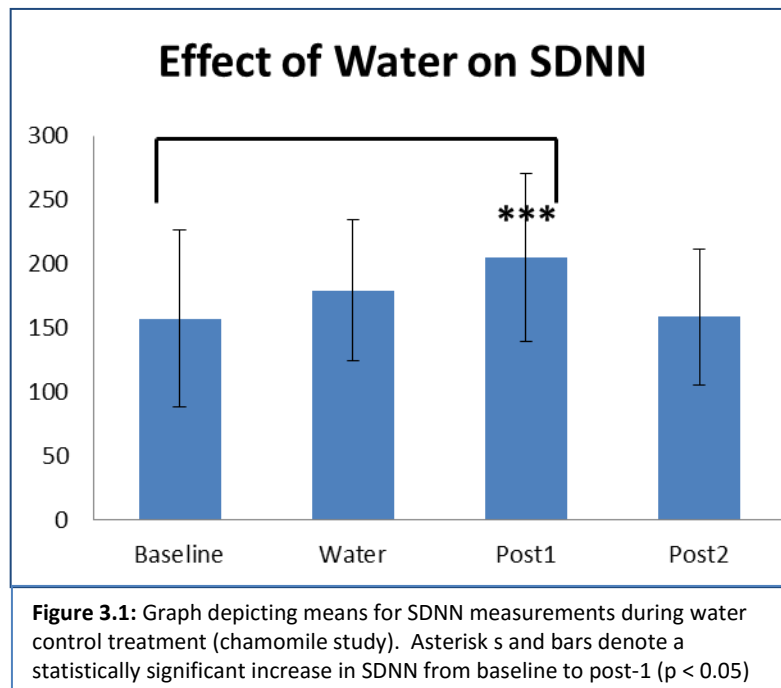
| Water      |                |                |                |                |
|------------|----------------|----------------|----------------|----------------|
|            | Baseline       | Water          | Post 1         | Post 2         |
| HR (bpm)   | 36.5 (± 9.2)   | 39.9 (± 8.1)   | 33.41 (± 7.8)  | 36.77 (± 8.0)  |
| SDNN (ms)  | 136.7 (± 84.5) | 195.4 (± 65.4) | 158.6 (± 67.7) | 111.3 (± 67.5) |
| RMSSD (ms) | 89.5 (± 44.8)  | 77.2 (± 51.8)  | 90.5 (± 57.7)  | 71.5 (± 51.6)  |
| % VLF      | 73.1 (± 15.8)  | 80.1 (± 19.0)  | 75.2 (± 18.4)  | 67.2 (± 20.2)  |
| % LF       | 19.4 (± 14.0)  | 14.4 (± 13.7)  | 17.6 (± 11.9)  | 22.1 (± 11.5)  |
| % HF       | 7.3 (± 4.3)    | 4.9 (± 5.5)    | 7.17 (± 8.1)   | 10.7 (± 9.7)   |

**Table 2.2** Means and standard deviations of HRV parameters measured during water control (lavender study). No statistical significance from baseline was found for any parameter.

### *Chamomile study*

In the chamomile study, only 2 of the 8 horses demonstrated an increase in RMSSD: Moon and Remi. Statistical significance was only found with the RMSSD parameters when both horses' data was removed: RMSSD significantly decreased from an overall mean of  $107.7 \pm 73.5$  ms to  $73.5 \pm 29.6$  ms ( $p = 0.031$ ). Statistical significance was also found with the water control; SDNN increased between baseline and post-1 measurements (Figure 3.1) from an overall mean of  $157.2 \pm 69.2$  ms to  $205.0 \pm 65.1$  ms ( $p = 0.034$ ). The overall means and standard deviations for each parameter in both the chamomile and water control treatments are listed in Table 3.1 and 3.2. Measurements for all the parameters in each individual horse are found in Table 3.3, 3.4, 3.5, and 3.6 in Appendix A.

### *Graph of statistically significant changes from baseline*



Tables of overall means and standard deviations for each HRV parameter

| Chamomile  |                |                |                |                |
|------------|----------------|----------------|----------------|----------------|
|            | Baseline       | Lavender       | Post 1         | Post 2         |
| HR (bpm)   | 38.9 (± 6.8)   | 37.2 (± 5.4)   | 37.5 (± 4.7)   | 38.8 (± 4.9)   |
| SDNN (ms)  | 199.9 (± 61.6) | 160.9 (± 55.7) | 165.7 (± 54.1) | 172.1 (± 50.7) |
| RMSSD (ms) | 101.9 (± 43.5) | 97.4 (± 57.7)  | 92.1 (± 37.6)  | 94.1 (± 20.7)  |
| % VLF      | 76.4 (± 8.8)   | 71.4 (± 24.3)  | 76.6 (± 11.3)  | 79.9 (± 11.5)  |
| % LF       | 17.4 (± 8.8)   | 19.3 (± 16.5)  | 15.0 (± 8.1)   | 12.1 (± 5.4)   |
| % HF       | 6.3 (± 2.6)    | 9.2 (± 9.0)    | 8.3 (± 6.5)    | 8.0 (± 9.8)    |

**Table 3.1:** Means and standard deviations of HRV parameters measured during chamomile treatment.

| Water      |                |                |                |                |
|------------|----------------|----------------|----------------|----------------|
|            | Baseline       | Water          | Post 1         | Post 2         |
| HR (bpm)   | 41.2 (± 7.8)   | 41.3 (± 9.6)   | 44.4 (± 6.8)   | 38.8 (± 5.3)   |
| SDNN (ms)  | 157.2 (± 69.2) | 179.4 (± 55.3) | 205.0 (± 65.1) | 158.9 (± 53.0) |
| RMSSD (ms) | 98.2 (± 47.3)  | 108.3 (± 51.2) | 109.5 (± 53.4) | 79.7 (± 28.4)  |
| % VLF      | 69.7 (± 15.4)  | 67.1 (± 19.7)  | 76.9 (± 12.8)  | 77.3 (± 9.2)   |
| % LF       | 42.9 (± 57.9)  | 25.9 (± 15.4)  | 16.6 (± 8.2)   | 17.4 (± 7.4)   |
| % HF       | 8.1 (± 5.7)    | 6.9 (± 4.9)    | 6.5 (± 5.5)    | 5.3 (± 2.7)    |

**Table 3.2:** Means and standard deviations of HRV parameters measured during water control treatment (chamomile study).

## Pilot Study Cortisol

Cortisol samples from Ely and Major both showed more significant changes from baseline when collected 20 minutes after baseline (as opposed to 5 or 10 minutes after). Cortisol levels for Ely dropped during chamomile treatment, and increased during water control treatment. Cortisol levels for Major increased with both chamomile and water control treatments; Major appeared to be stressed out in the pilot study with the procedure of collecting the salivary cortisol samples. During the chamomile study, this anxiety appeared to be resolved. The results from the salivary cortisol samples from the chamomile study are presently being analyzed at Salimetrics, LLC.

| Horse | Treatment      | Cortisol ( $\mu\text{g}/\text{dL}$ ) |
|-------|----------------|--------------------------------------|
| Ely   | Baseline       | 0.068                                |
| Ely   | Chamomile (5)  | 0.045                                |
| Ely   | Chamomile (10) | 0.046                                |
| Ely   | Chamomile (20) | 0.031                                |
| Major | Baseline       | 0.033                                |
| Major | Water (5)      | 0.044                                |
| Major | Water (10)     | 0.049                                |
| Major | Water (20)     | 0.047                                |
| Major | Baseline       | 0.025                                |
| Major | Chamomile (5)  | 0.033                                |
| Major | Chamomile (10) | 0.047                                |
| Major | Chamomile (20) | 0.050                                |
| Ely   | Baseline       | 0.043                                |
| Ely   | Water (5)      | 0.064                                |
| Ely   | Water (10)     | 0.087                                |
| Ely   | Water (20)     | 0.058                                |

**Table 4.1:** Salivary cortisol levels collected during the pilot study to measure the time course of changes in cortisol levels after exposure to treatment.

## Individual comparisons of HRV parameters from baseline:

### Pluto:

#### *Lavender study:*

- In both the water control and lavender treatments, HR increased slightly, but overall remained close to baseline measurements. With lavender, HR remained close to 42.0 *bpm* at baseline during treatment and post-1 (42.4 and 42.0 *bpm*), and increased slightly to 54.43 *bpm* during post-2; with water, HR increased slightly from 38.7 *bpm* at baseline to 39.2 and 40.0 *bpm* during treatment and post-1, and returned to near baseline during post-2 (38.4 *bpm*).
- SDNN was positively affected and increased in both treatments. With lavender, there was an increase from his baseline measurement of 89.3 *ms* to 103.4 *ms* during the administering of the humidified lavender; SDNN decreased to 65.5 *ms* during post-1, but increased again to 157.8 *ms* during post-2. With the water control, although SDNN increased, the amount of increase was modest: during the administering of the humidified water control, SDNN increased from a baseline measurement of 31.7 *ms* to 49.1 *ms* and continued to increase to 66.2 *ms* during post-1. His SDNN decreased from post-1 to 46.4 *ms* during post-2, overall still maintaining an increase from baseline.
- RMSSD was also positively affected in both treatments: with lavender, RMSSD increased slightly from 33.8 *ms* to 34.3 *ms* during treatment, decreased transiently to 30.6 *ms* during post-1, and increased to 44.5 *ms* during post-2. With water, his measurements increased from 23.6 *ms* to 25.8 *ms* during treatment, continued to increase to 30.6 *ms* during post-1, and decreased to 23.5 *ms* during post-2.



- In the lavender treatment, VLF decreased slightly during treatment from 77.0% at baseline to 76.2%, and more significantly during post-1 to 66.7%, but then recovered and increased during post-2 to 88.1%. With water, VLF increased from a baseline of 59.5% to 76.2%, 65.1%, and 81.5% during treatment, post-1, and post-2, respectively. Both the water and lavender treatments positively affected his VLF from baseline in comparison to post-2 measurements, though the amount of increase in VLF was greater for the water treatment.
- With lavender, LF increased slightly from a baseline of 21.5% to 22.5% during treatment, continued to increase to 29.3% during post-1, and decreased substantially to 8.5% during post-2. With water, LF decreased initially during treatment from 26.5% at baseline to 17.6%, recovered and increased during post-1 to 29.4%, and then decreased during post-2 to 14.7%.
- With lavender, HF decreased slightly during treatment from 1.5% to 1.2%, and increased to 4% and 3.4% during post-1 and post-2. With water, HF decreased from 14% at baseline to 6.1%, 5.5%, and 3.8% during treatment, post-1, and post-2.

*Chamomile study:*

\*\* Pluto's post-2 data for the chamomile treatment was lost during the extraction of the readings from the device and is not reported below.

- In both the water control and lavender treatments, HR only fluctuated slightly and overall remained close to baseline measurements. With chamomile, HR increased slightly from 47.2 *bpm* at baseline to 48.8 *bpm* during treatment, and decreased slightly to 46.5 *bpm* during post-1. With water, HR was 49.2 *bpm* at baseline, 49.1 *bpm* during treatment, 49.4 *bpm* during post-1, and decreased slightly to 48.8 *bpm* during post-2.

- SDNN was positively affected during the chamomile treatment, increasing from 103.2 *ms* at baseline to 127.1 *ms*; the increase was transient and decreased to 67.2 *ms* during post-1. SDNN also increased during the water treatment from 73.7 *ms* at baseline to 89.9 *ms*, decreasing to 63.7 *ms* during post-1, and increasing to 85.6 *ms* during post-2.
- RMSSD decreased from 45.58 *ms* at baseline to 25.6 and 30.5 *ms* during the chamomile treatment and post-1. With the water control, RMSSD only slightly increased from 28.7 *ms* at baseline to 32.2 *ms* during treatment, returning to near-baseline values during post-1 and post-2 (27.3 *ms* and 29.0 *ms*).
- VLF increased from 79.8% at baseline to 97.6% and 86.0% during the chamomile treatment and post-1, and decreased to 71.6% during post-2. With the water control, VLF decreased from a baseline of 79.1% to 56.7% and 57.2% during treatment and post-1, and recovered near baseline to 75.9% during post-2.
- LF decreased considerably with chamomile from a baseline of 34.1% to 4.6% during treatment, and began to recover to near-baseline during post-1 (22.3%). With water, LF increased during treatment and post-1 from 17.6% at baseline to 22.1% and 25.4%, and decreased to 12.9% during post-2.
- HF also decreased considerably with chamomile from a baseline of 5.2% to 0.6% during treatment, nearly recovering to baseline during post-1 (4.09%). With water, HF decreased slightly during treatment from 2.8% at baseline to 2.4%, and increased during post-1 and post-2 to 4.5% and 3.5%.

## Moon:

### *Lavender study:*

- HR only had minor fluctuations during both lavender and water treatments, overall staying relatively consistent. With lavender, HR was 37.5, 37.5, 36.9, and 40.3 *bpm*, and with water, 42.3, 38.8, 38.0, and 36.3 *bpm* during baseline, treatment, post-1, and post-2, respectively.
- SDNN decreased from baseline with both lavender and water during treatment, post-1, and post-2. With lavender, SDNN decreased from a baseline of 154.1 *ms* to 116.6, 101.4, and 77.9 *ms*, and with water, decreased from 224.2 *ms* to 185.4, 160.4, and 139.9 *ms*.
- RMSSD decreased with lavender, and increased with water. With lavender, RMSSD decreased from 164.4 *ms* to 154.3, 158.8, and 103.8 *ms*, and with water, increased from 162.2 *ms* to 184.6, 171.4, and 171.8 *ms* during treatment, post-1, and post-2.
- VLF decreased from baseline with both lavender and water during treatment, post-1, and post-2. With lavender, VLF decreased from a baseline of 62.9% to 41.5%, 24.7 %, and 40.2%, and with water, decreased from a baseline of 77.8% to 36.1%, 40.7%, and 33.7%. While both the water and lavender treatments negatively affected his VLF measurements from baseline vs. post-2, the lavender treatment had an attenuated decrease and yielded a higher post-2 VLF (relative to baseline) than the water treatment.
- LF increased initially during lavender treatment from a baseline of 18.0% to 19.0%, but decreased during post-1 and post-2 to 12.9% and 15.1%. With water, LF increased considerably from a baseline of 10% to 46.5%, 32.8%, and 38.3%.

- HF increased from baseline with both lavender and water during treatment, post-1, and post-2. With lavender, HF increased from 19.1% at baseline to 39.5%, 62.4%, and 44.6%, and with water, increased from 12.2% at baseline to 17.5%, 26.4%, and 28.0%.

*Chamomile study:*

\*\* Moon's post-2 data with the water control during the chamomile study was discarded due to erroneous readings from the device and is not reported below.

- HR decreased during the chamomile treatment from a baseline of 51.2 *bpm* to 39.7, 38.8, and 37.4 *bpm* during treatment, post-1, and post-2. With water, his HR increased slightly during treatment from 55.3 *bpm* to 60.1 *bpm*, but returned close to baseline during post-1 (56.9 *bpm*).
- SDNN measurements decreased with chamomile from 145.9 *ms* to 118.3, 160.0, and 122.9 *ms*. Similarly, with water, SDNN initially decreased from 192.6 *ms* to 126.9 *ms* from baseline to treatment, but increased to 236.1 *ms* during post-1.
- His RMSSD increased substantially with chamomile from 72.7 *ms* at baseline to 118.0, 134.7, and 132.2 *ms*. With water, RMSSD initially decreased from 139.1 *ms* to 55.0 *ms* between baseline and treatment, but recovered and increased to 193.5 *ms* during post-1.
- VLF decreased from 69.0% during baseline to 61.4%, 66.5%, and 61.3% during chamomile treatment, post-1, and post-2, respectively. With water, VLF increased from 51.2% at baseline to 70.7% and 61.3% during treatment and post-1.
- LF decreased from baseline with both chamomile and water. With chamomile, LF decreased from a baseline of 21.8% to 17.4%, 12.4%, and 10.5%, and with water, decreased from a baseline of 33.6% to 23.8% and 23.3%.

- HF increased with chamomile from a baseline of 9.2% to 21.2%, 21.1%, and 28.1% during treatment, post-1, and post-2. With water, HF decreased during treatment from a baseline of 15.2% to 5.5%, and recovered close to baseline at 15.4% during post-1.

### Dewie:

#### *Lavender study:*

- HR stayed relatively consistent with both lavender and water. With lavender, his measurements were 35.9, 35.8, 37.8, and 36.1 *bpm* during baseline, treatment, post-1, and post-2. With water, his measurements were 35.3, 35.3, 36.5, and 34.2 *bpm*.
- SDNN increased with lavender from a baseline of 91.0 *ms* to 126.4, 221.4, and 145.7 *ms*; with water, SDNN increased during treatment and post-1 from a baseline of 120.9 *ms* to 204.7 and 237.2 *ms*, but decreased considerably during post-2 to 40.7 *ms*.
- RMSSD was positively affected with the lavender, increasing from a baseline of 55.9 *ms* to 60.6, 139.6, and 78.2 *ms*. With water, RMSSD decreased to 64% during treatment from a baseline of 71.8%, increased during post-1 to 86.7%, but decreased again during post-2 to 42%.
- VLF increased slightly from a baseline of 78.0% to 78.3% during lavender treatment, decreased to 69.1% during post-1, and increased to 82.6% during post-2. With water, VLF increased from a baseline of 69.8% to 95.2% and 88.4% during treatment and post-1, and decreased during post-2 to 45.3%.
- LF increased from a baseline of 14.3% to 15.8% and 25.9% during lavender treatment and post-1, but decreased to 11.8% during post-2. With water, LF decreased considerably from a baseline of 23.5% to 3.7% and 9.6% during treatment and post-1, but recovered and increased to 31.2% during post-2.

- HF decreased from a baseline of 7.6% to 5.9%, 5.1%, and 5.6% during lavender treatment, post-1, and post-2; with water, HF decreased from a baseline of 6.7% to 1.1% and 2.0% during treatment and post-1, but increased considerably to 23.5% during post-2.

*Chamomile study:*

- HR stayed relatively close to baseline with chamomile and water. With chamomile, HR measurements were 33.5, 32.6, 33.3, and 36.0 *bpm*, and with water, 37.2, 45.3, 48.3, and 38.0 *bpm* during baseline, treatment, post-1, and post-2.
- SDNN decreased with both chamomile and water. With chamomile, SDNN decreased considerably from a baseline of 239.4 *ms* to 93.7 *ms* during treatment, recovering to 186.8 and 215.1 *ms* during post-1 and post-2. With water, the change in SDNN was modest, decreasing from a baseline of 232.1 *ms* to 228.3, 213.5, and 208.7 *ms*.
- RMSSD decreased from a baseline of 94.0 *ms* to 70.5 and 77.8 *ms* during lavender treatment and post-1, and increased to 108.3 *ms* during post-2. With water, RMSSD decreased from baseline during treatment, post-1, as well as post-2 (159.4, 96.5, 87.4, and 107.0 *ms*).
- VLF decreased with chamomile from a baseline of 82.0% to 70.9%, 67.5%, and 78.0% during baseline, treatment, post-1, and post-2. With water, VLF increased from 61.9% to 84.8%, 78.1%, and 76.2%.
- LF increased with chamomile and decreased with water. With chamomile, LF increased from a baseline of 13.8% to 21.2%, 18.8%, and 20.1%. With water, LF decreased from a baseline of 29.6% to 11.5%, 18.32%, and 18.37%.

- HF increased from a baseline of 4.2% to 7.9% and 13.7% during chamomile treatment and post-1, and decreased during post-2 to 1.9%. With water, HF decreased from a baseline of 8.5% to 3.74%, 3.7%, and 5.4%.

### **Bianca:**

#### *Lavender study:*

- HR stayed relatively consistent with lavender, both increasing and decreasing slightly from a baseline of 38.7 *bpm* to 37.6, 38.8, and 39.4 *bpm*. With water, HR decreased from a baseline of 44.8 *bpm* to 33.3, 32.5, and 23.8 *bpm*.
- SDNN increased with lavender and decreased with water. From a baseline of 138.9 *ms*, SDNN increased to 155.0 *ms* during lavender treatment, 155.9 *ms* during post-1, and 183.74 *ms* during post-2. With water, SDNN decreased from a baseline of 236.9 *ms* to 171.4, 191.5, and 82.3 *ms*, considerably decreasing during post-2.
- RMSSD with lavender increased during treatment and post-1 from 109.1 *ms* to 131.5 and 131.9 *ms*, and then recovered close to baseline during post-2 to 109.7 *ms*. With water, RMSSD initially decreased slightly during treatment from 69.2 *ms* to 66.5 *ms*, increased considerably during post-1 to 92.9 *ms*, and then decreased to near baseline during post-2 to 66.9 *ms*.
- VLF with lavender increased slightly from 72.7% to 77.1% during treatment, but decreased to 68.4% and 70.1% during post-1 and post-2. With water, VLF decreased during treatment from 91.8% to 84.4%, recovered close to baseline during post-1 to 90.4%, and decreased during post-2 to 82.3%. Both the water and lavender treatments negatively affected her VLF measurements from baseline vs. post-2; however, similarly

to Moon, the amount of decrease in VLF for post-2 was less with the lavender than it was for the water treatment.

- LF with lavender decreased initially during treatment from 10.4% to 9.2%, but then increased to 16.3% and 12.5% during post-1 and post-2. With water, LF increased during treatment from 5.8% to 9.6%, decreased near baseline during post-1 to 5.6%, and increased during post-2 to 10.9%.
- HF with lavender decreased during treatment from 16.9% to 13.7%, began to recover in post-1 to 15.4%, and increased during post-2 to 17.3%. With water, HF increased during treatment from 2.4% to 6.0%, decreased during post-1 to 4.0%, and increased during post-2 to 6.8%.

*Chamomile study:*

- HR again remains relatively consistent, with minor fluctuations from baseline in both chamomile and water treatments. With chamomile, HR measurements decreased slightly from a baseline of 39.8 *bpm* to 34.6, 32.8, and 33.6 *bpm*. With water, HR slightly increased from 35.0 *bpm* to 39.0, 41.0, and 38.7 *bpm*.
- SDNN decreased with chamomile, while it increased with water. With chamomile, SDNN decreased from a baseline of 273.6 *ms* to 233.6, 116.7, and 93.2 *ms*. With water, SDNN increased from a baseline of 170.9 *ms* to 219.5, 210.5, and 228.2 *ms*.
- RMSSD decreased with chamomile, while RMSSD increased with water. With chamomile, RMSSD decreased considerably from a baseline of 183.8 *ms* to 86.1, 61.7, and 68.5 *ms*. With water, RMSSD increased from a baseline of 106.6 *ms* to 174.7 and 165.2 *ms* during treatment and post-1, and recovered near baseline to 105.0 *ms* during post-2.



- VLF with chamomile increased from a baseline of 79.8% to 97.6% and 86.0% during treatment and post-1, and decreased during post-2 to 71.6%. With water, VLF decreased from 79.1% to 56.7% and 57.2% during treatment and post-1, but recovered close to baseline during post-2 to 75.9%.
- LF with chamomile decreased during treatment from 9.9% to 1.4%, recovered during post-1 to 9.1%, and increased during post-2 to 15.9%. With water, LF increased during treatment and post-1 from 18.4% to 30.9% and 27.6%, and decreased during post-2 to 14.7%
- HF with chamomile decreased during treatment and post-1 from 10.3% to 1.0% and 4.9%, and increased during post-2 to 12.6%. With water, HF increased from 2.5% to 12.4%, 15.2%, and 9.4%.

### Remi:

#### *Lavender study:*

- HR stayed relatively consistent with lavender, fluctuating only slightly from baseline; the HR measurements were 36.0, 34.6, 41.0, and 34.0 *bpm* for baseline, treatment, post-1, and post-2. With water, HR increased from an initially low baseline of 20.5 *bpm* to 38.5, 47.7, and 47.6 *bpm*.
- SDNN decreased with lavender from a baseline of 304.9 *ms* to 286.4, 260.2, and 60.2 *ms*. With water, SDNN increased considerably during treatment from a baseline of 84.9 *ms* to 243.6 *ms*, and then decreased to 54.5 and 45.1 *ms* during post-1 and post-2.
- RMSSD increased initially during the lavender treatment from 80.5 *ms* to 113.0 *ms*, but then decreased to 46.1 and 26.5 *ms* during post-1 and post-2. With water, RMSSD decreased from 94.5 *ms* at baseline to 44.5, 16.3, and 18.7 *ms*.

- VLF initially decreased with lavender from a baseline of 99.0% to 85.3%, but then recovered to near baseline during post-1 and post-2 (99.2% and 98.7%). With water, VLF initially increased during treatment and post-1 from 45.3% to 88.2% and 93.7%, but then recovered and decreased to near baseline during post-2 (57.0%).
- LF increased considerably with lavender treatment from a baseline of 0.7% to 10.9%, but then recovered and decreased to near baseline during post-1 and post-2 (99.2% and 98.7%). With water, LF decreased from a comparatively high baseline of 47.7% to 10.4% and 4.9% during treatment and post-1, and recovered to near baseline during post-2 to 34.4%.
- HF increased with lavender treatment from a baseline of 0.25% to 3.8%, and decreased near baseline during post-1 and post-2 (0.29% and 0.32%). With water, HF decreased from a baseline of 6.9% to 1.39% and 1.43% during treatment and post-1, and increased during post-2 to 8.6%.

*Chamomile study:*

- HR increased with chamomile, though stays relatively consistent, with a baseline of 32.6 *bpm* and measurements of 35.8, 36.3, and 35.1 *bpm* during treatment, post-1, and post-2. With water, HR likewise does not fluctuate significantly from baseline, with measurements of 36.7, 33.0, 37.0, and 34.5 *bpm*.
- SDNN increased during both the chamomile and water treatment. With chamomile, SDNN increased from a baseline of 180.2 *ms* to 228.3 *ms* during treatment, and recovered near baseline to 182.1 and 182.4 *ms* during post-1 and post-2. With water, SDNN increased from a baseline of 129.8 *ms* to 250.0, 246.4, and 183.6 *ms*.

- RMSSD with chamomile increased during treatment and post-1 from a baseline of 96.2 *ms* to 220.3 and 111.3 *ms*, and decreased to near baseline during post-2 to 91.9 *ms*. With water, RMSSD increased from a baseline of 94.9 *ms* to 136.26, 122.96, and 104.63 *ms*.
- VLF decreased during chamomile treatment from a baseline of 70.9 to 44.3%, and increased during post-1 and post-2 to 13.2% and 10.1%. With water, VLF increased from a baseline of 57.4% to 85.9%, 87.8%, and 70.2%.
- LF increased during chamomile treatment from a baseline of 24.6% to 40.5%, and decreased during post-1 and post-2 to 13.2% and 10.1%. With water, LF decreased from a baseline of 28.8% to 10.0%, 8.6%, and 24.6%.
- HF increased during the chamomile treatment from a baseline of 4.5% to 15.2%, but decreased during post-1 and post-2 to 4.0% and 1.8%. With water, HF decreased from a baseline of 13.8% to 4.07%, 3.6%, and 5.2%.

### Anna:

#### *Lavender study:*

- HR did not fluctuate significantly from the baseline measurement in both lavender and water treatments. With lavender, the HR measurements were 30.9, 30.2, 30.9, and 40.9 *bpm*, and with water, 32.0, 31.0, 31.7, and 30.5 *bpm* during baseline, treatment, post-1, and post-2.
- SDNN initially decreased slightly from a baseline of 169.6 *ms* to 162.4 *ms* during the lavender treatment, and increased to 205.6 and 184.6 *ms* during post-1 and post-2. With water, SDNN increased from a baseline of 171.9 *ms* to 219.0, 217.7, and 185.6 *ms*.
- RMSSD during lavender treatment increased from a baseline of 102.9 *ms* to 138.12 *ms*, decreased during post-1 to 84.3 *ms*, and increased to 141.8 *ms* during post-2. With water,

the opposite was observed: RMSSD decreased during treatment to 120.7 *ms* from a baseline of 146.2 *ms*, increased to 150.4 *ms* during post-1, and decreased to 121.5 *ms* during post-2.

- VLF decreased during the lavender treatment from a baseline of 85.3% to 79.2%, 80.1%, and 72.2%. With water, VLF increased from a baseline of 58.6% to 82.2% and 75.2% during treatment and post-1, and decreased close to baseline during post-2 (66.3%).
- LF increased during lavender treatment from a baseline of 12.0% to 12.6%, 14.4%, and 23.7%. With water, LF decreased from a baseline of 23.5% to 13.7% and 18.4% during treatment and post-1, and recovered and increased near baseline to 24.2% during post-2.
- HF increased during lavender treatment from a baseline of 2.7% to 8.1%, 5.6%, and 4.1%. With water, HF decreased from a baseline of 6.9% to 4.1% during treatment, recovered to 6.4% during post-1, and increased to 9.6% during post-2.

*Chamomile study:*

- HR does not fluctuate significantly from baseline in both chamomile and water treatments. The HR measurements during baseline, treatment, post-1, and post-2 were 33.9, 37.4, 38.1, and 36.0 *bpm* with chamomile, and 34.0, 32.9, 36.9, and 41.5 *bpm* with water.
- SDNN increased from a baseline of 204.0 *ms* to 216.1 *ms* during chamomile treatment, decreased to 198.9 *ms* during post-1, and increased during post-2 to 220.5 *ms*. With water, SDNN decreased from a baseline of 264.3 *ms* during baseline to 192.9 *ms* during treatment, increased during post-1 to 283.6 *ms*, and decreased during post-2 to 170.2 *ms*.

- RMSSD decreased during both the chamomile and water treatments. RMSSD measurements during baseline, treatment, post-1, and post-2 were 105.5, 75.4, 69.6, and 84.5 *ms* with chamomile, and 142.0, 85.5, 125.4, and 66.4 *ms* with water.
- VLF increased with chamomile from a baseline of 78.8% to 87.0%, 92.8%, and 90.5%. With water, VLF decreased from a baseline of 88.3% to 79.6% and 82.6% during treatment and post-1, and recovered near baseline during post-2 to 89.3%.
- LF decreased with chamomile from a baseline of 15.2% to 11.9%, 6.2%, and 7.1% during treatment, post-1, and post-2. With water, LF increased from a baseline of 8.8% to 18.7% and 13.7% during treatment and post-1, and recovered near baseline during post-2 to 8.96%.
- HF decreased with chamomile from a baseline of 6.0% to 1.04%, 0.98%, and 2.4%. With water, HF decreased from a baseline of 3.0% to 1.76%, during treatment, increased during post-1 to 3.7%, and decreased to 1.8% during post-2.

### Flash:

#### *Lavender study:*

- HR does not fluctuate significantly from baseline in both lavender and water treatments. HR measurements for baseline, treatment, post-1, and post-2 were 40.5, 37.7, 38.9, and 45.8 *bpm* for lavender, and 49.0, 56.2, 51.5, and 49.3 *bpm* for water.
- SDNN decreased with lavender from a baseline of 190.3 *ms* to 99.7, 149.3, and 165.6 *ms*. With water, SDNN increased from a baseline of 200.4 *ms* to 244.0 *ms* during treatment, recovered near baseline during post-1 to 199.8 *ms*, and increased during post-2 to 213.4 *ms*.

- RMSSD increased with lavender from a baseline of 60.9 *ms* to 89.9, 63.4, and 93.6 *ms*. With water, RMSSD slightly increased from a baseline of 70.9 *ms* to 71.0 *ms* during treatment, and decreased during post-1 and post-2 to 65.7 and 66.0 *ms*.
- VLF decreased with lavender and increased with water. With lavender, VLF decreased from a baseline of 86.4% to 54.0%, 79.1%, and 77.6%. With water, VLF increased from a baseline of 81.7% to 91.3%, 87.0%, and 89.8%.
- LF increased initially during the lavender treatment and post-1 to 35.4% and 15.6% from a baseline of 11.8%, but recovered and decreased during post-2 to 9.6%. With water, LF decreased from a baseline of 10.4% to 6.2%, 9.1%, and 8.6%.
- HF increased with lavender and decreased with water. With lavender, HF increased from a baseline of 1.8% to 10.6% during treatment, 5.4% during post-1, and 12.8% during post-2. With water, HF decreased from a baseline of 7.9% to 2.4%, 3.9%, and 1.6%.

*Chamomile study:*

- HR does not fluctuate significantly from baseline in both chamomile and water treatments. HR measurements for baseline, treatment, post-1, and post-2 were 37.2, 37.3, 41.1, and 38.3 *bpm* for chamomile, and 36.7, 37.1, 41.6, and 37.3 *bpm* for water.
- SDNN decreased during chamomile treatment to 132.9 *ms* from a baseline of 173.8 *ms*, and increased during post-1 and post-2 to 246.4 and 217.9 *ms*. With water, SDNN increased from a baseline of 93.7 *ms* to 141.5, 207.8, and 125.5 *ms*.
- RMSSD decreased from a baseline of 66.8 *ms* to 75.1 *ms* during chamomile treatment, and increased to 125.4 and 81.8 *ms* during post-1 and post-2. With water, RMSSD increased from a baseline of 65.9 *ms* to 116.7, 74.1, and 74.9 *ms*.

- VLF decreased slightly during chamomile treatment and post-1 to 84.6% and 83.5% from a baseline of 87.9%, and increased during post-2 to 93.4%. With water, VLF increased from a baseline of 53.3% to 56.5%, 91.7%, and 62.1%.
- LF increased from a baseline of 9.4% to 10.4% during chamomile treatment, and decreased to 8.4% and 5.1% during post-1 and post-2. With water, LF increased slightly from a baseline of 31.9% to 32.3% during treatment, decreased to 6.9% during post-1, and recovered near baseline to 29.8% during post-2.
- HF increased with lavender during treatment and post-1 to 5.0% and 8.2% from a baseline of 2.7%, and decreased during post-2 to 1.5%. With water, HF decreased from a baseline of 14.8% to 11.3%, 1.4%, and 8.1%.

### Daphne:

#### *Lavender study:*

- HR stayed relatively consistent with lavender, with measurement of 31.7, 32.7, 30.9, and 29.8 *bpm*. With water, HR increased during treatment to 46.6 *bpm* from a baseline of 29.7 *bpm*, and then decreased, recovering near baseline during post-1 and post to 29.5 and 27.9 *bpm*.
- SDNN during lavender and water both transiently increased during treatment. With lavender, SDNN increased from a baseline of 246.8 *ms* to 311.9 *ms* during treatment and 288.0 *ms* during post-1, and decreased to 142.5 *ms* during post-2. With water, SDNN increased from a baseline of 220.7 *ms* to 245.7 *ms* during treatment and then decreased during post-1 and post-2 to 141.4 and 137.3 *ms*.
- RMSSD increased initially from a baseline of 84.8 *ms* to 112.5 and 94.7 *ms* during chamomile treatment and post-1, but decreased during post-2 to 66.6 *ms*. With water,

RMSSD decreased from a baseline of 77.6 *ms* to 40.8 *ms* during treatment, increased during post-1 to 109.5 *ms*, and decreased during post-2 to 62.1 *ms*.

- VLF decreased from a baseline of 88.2% to 81.9% during chamomile treatment, recovered during post-1 to 89.9%, and decreased during post-2 to 79.4%. With water, VLF increased slightly during treatment from a baseline of 90.7% to 91.7%, and decreased during post-1 and post-2 to 61.4% and 81.6%.
- LF increased initially from a baseline of 10.4% to 15.9% during chamomile treatment, decreased during post-1 to 8.9%, and increased during post-2 to 19.0%. With water, LF decreased slightly from a baseline of 7.9% to 7.2% during treatment and increased during post-1 and post-2 to 30.9% and 14.6%.
- HF increased slightly during lavender treatment from a baseline of 1.5% to 2.2% and then recovered near baseline during post-1 and post-2 to 1.2% and 1.6%. With water, HF decreased during treatment from a baseline of 1.4% to 0.86%, and increased during post-1 and post-2 to 7.7% and 3.8%.

*Chamomile study:*

\*\* Daphne was relocated to another barn and could not be used during the chamomile study. Another horse, Major, was substituted in her place.

### **Major:**

*Chamomile study:*

\*\* Edited Major's baseline and post-1 because erroneous data collection from device, only selected for the last half of recording.



- HR did not fluctuate much from baseline during both chamomile and water treatments. HR measurements for baseline, treatment, post-1, and post-2 were 36.0, 31.5, 33.7, and 35.3 *bpm* with chamomile, and : 45.4, 33.8, 44.0, and 32.6 *bpm* with water.
- SDNN decreased with chamomile and increased with water. With chamomile, SDNN decreased from a baseline of 278.6 *ms* to 137.0, 168.3, and 152.9 *ms*. With water, SDNN increased from a baseline of 100.4 *ms* to 186.4, 178.3, and 110.6 *ms*.
- RMSSSD decreased with chamomile and increased with water. With chamomile, RMSSSD decreased from a baseline of 142.5 *ms* to 116.8, 126.1, and 91.7 *ms*. With water, RMSSSD increased from a baseline of 49.2 *ms* to 169.8, 80.0, and 71.0 *ms*.
- VLF decreased from a baseline of 81.8% to 30.7%, 60.4%, and 76.5% with chamomile treatment, decreasing considerably during treatment and gradually approaching baseline during post-1 and post-2. With water, VLF similarly decreased from a baseline of 87.3% to 27.3% during treatment, recovering near baseline during post-1 and post-2 to 87.2% and 83.9%.
- LF increased with chamomile from a baseline of 10.1% to 47.3%, 30.0%, and 15.8%. With water, LF increased considerably from a baseline of 8.7% to 58.4% during treatment, recovered to baseline during post-1 to 8.7%, and increased slightly to 12.2% during post-2.
- HF increased considerably with chamomile from a baseline of 8.0% to 22.0% during treatment, recovered near baseline during post-1 to 9.7%, and decreased during post-2 to 7.7%. With water, HF similarly increased transiently from a baseline of 4.1% to 14.3% during treatment, recovered to baseline during post-1 at 4.1%, and decreased slightly during post-2 to 3.9%.

## Discussion:

We predicted to see significant increases in very low frequency (VLF) rhythms and RMSSD time domain (which measures vagus nerve mediated autonomic control) during essential oil treatments compared to control.

Of the 8 horses, lavender only increased VLF for 2 horses: Pluto and Dewie, and for Pluto, both treatments increased his VLF. For 3 of the horses (Moon, Bianca, and Daphne), although VLF decreased for both treatments, the decrease was attenuated with the lavender treatment (resulting in a higher VLF measurement with lavender in comparison to water). While the VLF findings varied and deviated from our hypothesis, RMSSD results were in line with our predictions. Of the 8 horses, 7 demonstrated improved RMSSD scores with lavender; Pluto was the only horse whose RMSSD decreased. RMSSD showed no statistical significance from baseline in any parameter of HRV with the water treatment. Of the 7 horses that had positive effects with lavender, it was interesting to note that 3 of them also demonstrated positive increases with water as well.

During the chamomile study, VLF increased for 4 of the horses: Bianca, Pluto, Flash, and Anna. RMSSD only increased for 2 horses: Moon and Remi. When the data for these two horses were removed, there was statistical significance from baseline for RMSSD to decrease. Additionally, during the water control, there was a statistically significant increase from baseline in SDNN, a parameter which describes the amplitude of HRV (Baldwin, Lecture 5). This increase is likely due to the effect of respiration on the control of heart rate; even with a diffuser expelling humidified air, their respiration alone may be contributing to this improved parameter.

Although the VLF findings haven't supported our hypothesis, it is encouraging to discover that RMSSD measurements appear to respond positively with lavender. VLF itself is

still not fully understood, and there are likely other factors which contribute to why VLF may fluctuate. The decrease in VLF may be due to a shift towards more parasympathetic tone, as reflected with the increased RMSSD in the lavender study, and the increase in VLF was reflected with a decrease in RMSSD in the chamomile study.

One possible explanation for why lavender and chamomile treatments appear to have different effects in the horses overall may have to do with the molecular basis of the active constituents in both essential oils. One study determined that the mechanism of action for lavender involves serotonergic rather than GABA/benzodiazepine neurotransmission (Chioca et al, 2013), while another study discovered that for lemongrass essential oils, the GABAergic system is more involved (Costa et al , 2011). Further research into the molecular basis of the essential oils and the pharmacological activity they produce is still being explored.

This experiment is ongoing and further data will be collected to test for the reproducibility of the results obtained. Behavioral analysis of the videos recorded during the experiment for each of the horses will be performed to determine whether there is any significant trends in behavior changes that occur with aromatherapy. Additionally, the analyzed salivary cortisol data will provide useful insight into how aromatherapy may impact stress levels in horses. Horses are equipped with acute senses and can become easily stressed. Repeated acute stress, as well as chronic stress, leads to both behavioral and medical ailments such as inflammation, digestive disorders, and immunosuppression. Therefore minimizing stress is critical to maintaining equine health.

## Appendix A

Tables: 2.3 and 2.4

| Lavender Data |                          |                          |                       |                       |                           |                           |                        |                        |                            |                            |                         |                         |
|---------------|--------------------------|--------------------------|-----------------------|-----------------------|---------------------------|---------------------------|------------------------|------------------------|----------------------------|----------------------------|-------------------------|-------------------------|
|               | HR:<br>(bpm)<br>Baseline | HR:<br>(bpm)<br>Lavender | HR:<br>(bpm)<br>Post1 | HR:<br>(bpm)<br>Post2 | SDNN:<br>(ms)<br>Baseline | SDNN:<br>(ms)<br>Lavender | SDNN:<br>(ms)<br>Post1 | SDNN:<br>(ms)<br>Post2 | RMSSD:<br>(ms)<br>Baseline | RMSSD:<br>(ms)<br>Lavender | RMSSD:<br>(ms)<br>Post1 | RMSSD:<br>(ms)<br>Post2 |
| Pluto         | 41.95                    | 42.38                    | 42.01                 | 53.43                 | 89.30                     | 103.40                    | 65.50                  | 157.80                 | 33.80                      | 34.30                      | 30.60                   | 44.50                   |
| Moon          | 37.53                    | 37.52                    | 36.88                 | 40.30                 | 154.06                    | 116.6                     | 101.40                 | 77.90                  | 164.37                     | 154.30                     | 158.80                  | 103.80                  |
| Dewie         | 35.90                    | 35.81                    | 37.78                 | 36.12                 | 91.02                     | 126.36                    | 221.40                 | 145.70                 | 55.86                      | 60.65                      | 139.46                  | 78.20                   |
| Bianca        | 38.74                    | 37.59                    | 38.827                | 39.37                 | 138.89                    | 154.96                    | 155.88                 | 183.74                 | 109.12                     | 131.52                     | 131.88                  | 109.70                  |
| Remi          | 35.98                    | 34.60                    | 40.972                | 33.96                 | 304.88                    | 286.43                    | 260.22                 | 60.18                  | 80.50                      | 113.04                     | 46.12                   | 26.45                   |
| Anna          | 30.94                    | 30.23                    | 30.873                | 40.87                 | 169.56                    | 162.35                    | 205.57                 | 184.56                 | 102.90                     | 138.12                     | 84.26                   | 141.83                  |
| Flash         | 40.60                    | 37.65                    | 38.93                 | 45.84                 | 190.33                    | 99.74                     | 149.30                 | 165.64                 | 60.87                      | 89.89                      | 63.35                   | 93.64                   |
| Daphne        | 31.66                    | 32.74                    | 30.91                 | 29.77                 | 246.81                    | 311.92                    | 287.97                 | 142.51                 | 84.75                      | 112.53                     | 94.70                   | 66.58                   |

**Table 2.3:** Data values of HRV parameters measured during lavender treatment

| Lavender Data |                      |                      |                   |                   |                     |                     |                  |                  |                     |                     |                  |                  |
|---------------|----------------------|----------------------|-------------------|-------------------|---------------------|---------------------|------------------|------------------|---------------------|---------------------|------------------|------------------|
|               | VLF: (%)<br>Baseline | VLF: (%)<br>Lavender | VLF: (%)<br>Post1 | VLF: (%)<br>Post2 | LF: (%)<br>Baseline | LF: (%)<br>Lavender | LF: (%)<br>Post1 | LF: (%)<br>Post2 | HF: (%)<br>Baseline | HF: (%)<br>Lavender | HF: (%)<br>Post1 | HF: (%)<br>Post2 |
| Pluto         | 77.00                | 76.30                | 66.70             | 88.10             | 21.50               | 22.50               | 29.30            | 8.50             | 1.50                | 1.20                | 4.00             | 3.40             |
| Moon          | 62.87                | 41.50                | 24.70             | 40.20             | 17.98               | 19.00               | 12.90            | 15.10            | 19.14               | 39.50               | 62.40            | 44.60            |
| Dewie         | 78.05                | 78.34                | 69.07             | 82.63             | 14.32               | 15.80               | 25.86            | 11.76            | 7.63                | 5.86                | 5.07             | 5.61             |
| Bianca        | 72.70                | 77.11                | 68.35             | 70.07             | 10.44               | 9.23                | 16.29            | 12.64            | 16.86               | 13.66               | 15.36            | 17.29            |
| Remi          | 99.01                | 85.34                | 99.23             | 98.73             | 0.74                | 10.85               | 0.49             | 0.95             | 0.25                | 3.81                | 0.29             | 0.32             |
| Anna          | 85.27                | 79.23                | 80.08             | 72.23             | 11.99               | 12.63               | 14.36            | 23.67            | 2.75                | 8.14                | 5.56             | 4.10             |
| Flash         | 86.39                | 53.98                | 79.06             | 77.55             | 11.83               | 35.38               | 15.57            | 9.60             | 1.78                | 10.64               | 5.37             | 12.84            |
| Daphne        | 88.17                | 81.89                | 89.94             | 79.41             | 10.37               | 15.90               | 8.89             | 18.95            | 1.46                | 2.22                | 1.17             | 1.64             |

**Table 2.4:** Data values of HRV parameters measured during lavender treatment

Tables: 2.5 and 2.6

| Water Treatment |                          |                       |                       |                       |                           |                        |                        |                        |                            |                         |                         |                         |
|-----------------|--------------------------|-----------------------|-----------------------|-----------------------|---------------------------|------------------------|------------------------|------------------------|----------------------------|-------------------------|-------------------------|-------------------------|
|                 | HR:<br>(bpm)<br>Baseline | HR:<br>(bpm)<br>Water | HR:<br>(bpm)<br>Post1 | HR:<br>(bpm)<br>Post2 | SDNN:<br>(ms)<br>Baseline | SDNN:<br>(ms)<br>Water | SDNN:<br>(ms)<br>Post1 | SDNN:<br>(ms)<br>Post2 | RMSSD:<br>(ms)<br>Baseline | RMSSD:<br>(ms)<br>Water | RMSSD:<br>(ms)<br>Post1 | RMSSD:<br>(ms)<br>Post2 |
| Pluto           | 38.74                    | 39.20                 | 39.97                 | 38.44                 | 31.70                     | 49.10                  | 66.20                  | 46.40                  | 23.60                      | 25.80                   | 30.60                   | 23.50                   |
| Moon            | 42.29                    | 38.77                 | 37.97                 | 36.36                 | 224.20                    | 185.40                 | 160.40                 | 139.90                 | 162.20                     | 184.60                  | 171.40                  | 171.80                  |
| Dewie           | 35.29                    | 35.29                 | 36.48                 | 34.23                 | 120.90                    | 204.70                 | 237.20                 | 40.70                  | 71.80                      | 64.00                   | 86.70                   | 42.00                   |
| Bianca          | 44.75                    | 33.29                 | 32.48                 | 29.79                 | 236.90                    | 171.40                 | 191.50                 | 82.30                  | 69.20                      | 66.50                   | 92.90                   | 66.90                   |
| Remi            | 20.48                    | 38.49                 | 47.73                 | 47.64                 | 84.86                     | 243.60                 | 54.52                  | 45.09                  | 94.51                      | 44.50                   | 16.53                   | 18.675                  |
| Anna            | 31.97                    | 30.98                 | 31.74                 | 30.49                 | 171.90                    | 218.95                 | 217.73                 | 185.58                 | 146.19                     | 120.66                  | 150.35                  | 121.52                  |
| Flash           | 48.99                    | 56.18                 | 51.49                 | 49.32                 | 200.43                    | 243.98                 | 199.82                 | 213.38                 | 70.90                      | 71.02                   | 65.68                   | 65.964                  |
| Daphne          | 29.68                    | 46.60                 | 29.45                 | 27.93                 | 22.69                     | 245.73                 | 141.42                 | 137.27                 | 77.61                      | 40.82                   | 109.47                  | 62.054                  |

**Table 2.5:** Data values of HRV parameters measured during water treatment (lavender study)

| Water Treatment |                      |                   |                   |                   |                     |                  |                  |                  |                     |                  |                  |                  |
|-----------------|----------------------|-------------------|-------------------|-------------------|---------------------|------------------|------------------|------------------|---------------------|------------------|------------------|------------------|
|                 | VLF: (%)<br>Baseline | VLF: (%)<br>Water | VLF: (%)<br>Post1 | VLF: (%)<br>Post2 | LF: (%)<br>Baseline | LF: (%)<br>Water | LF: (%)<br>Post1 | LF: (%)<br>Post2 | HF: (%)<br>Baseline | HF: (%)<br>Water | HF: (%)<br>Post1 | HF: (%)<br>Post2 |
| Pluto           | 59.50                | 76.20             | 65.10             | 81.50             | 26.50               | 17.60            | 29.40            | 14.70            | 14.00               | 6.10             | 5.50             | 3.80             |
| Moon            | 77.80                | 36.10             | 40.70             | 33.70             | 10.00               | 46.50            | 32.80            | 38.30            | 12.20               | 17.50            | 26.40            | 28.00            |
| Dewie           | 69.80                | 95.20             | 88.40             | 45.30             | 23.50               | 3.70             | 9.60             | 31.20            | 6.70                | 1.10             | 2.00             | 23.50            |
| Bianca          | 91.80                | 84.40             | 90.40             | 82.30             | 5.80                | 9.60             | 5.60             | 10.90            | 2.40                | 6.00             | 4.00             | 6.80             |
| Remi            | 45.325               | 88.20             | 93.68             | 57.01             | 47.73               | 10.41            | 4.88             | 34.42            | 6.95                | 1.39             | 1.43             | 8.57             |
| Anna            | 68.617               | 82.21             | 75.22             | 66.28             | 23.45               | 13.71            | 18.35            | 24.19            | 6.93                | 4.08             | 6.43             | 9.60             |
| Flash           | 81.639               | 91.32             | 86.96             | 89.80             | 10.41               | 6.23             | 9.09             | 8.55             | 7.95                | 2.44             | 3.94             | 1.65             |
| Daphne          | 90.662               | 91.97             | 61.44             | 81.58             | 7.91                | 7.17             | 30.90            | 14.60            | 1.43                | 0.86             | 7.67             | 3.82             |

**Table 2.6:** Data values of HRV parameters measured during water treatment (lavender study)

Tables: 3.3 and 3.4

| Chamomile Treatment |                    |                     |                 |                 |                     |                      |                  |                  |                      |                       |                   |                   |
|---------------------|--------------------|---------------------|-----------------|-----------------|---------------------|----------------------|------------------|------------------|----------------------|-----------------------|-------------------|-------------------|
|                     | HR: (bpm) Baseline | HR: (bpm) Chamomile | HR: (bpm) Post1 | HR: (bpm) Post2 | SDNN: (ms) Baseline | SDNN: (ms) Chamomile | SDNN: (ms) Post1 | SDNN: (ms) Post2 | RMSSD: (ms) Baseline | RMSSD: (ms) Chamomile | RMSSD: (ms) Post1 | RMSSD: (ms) Post2 |
| Dewie               | 33.49              | 32.62               | 33.25           | 35.98           | 239.40              | 93.70                | 186.80           | 215.10           | 94.00                | 70.50                 | 77.80             | 108.30            |
| Bianca              | 39.79              | 34.61               | 32.75           | 33.55           | 273.62              | 233.55               | 116.65           | 93.159           | 183.83               | 86.10                 | 61.72             | 68.51             |
| Moon                | 51.16              | 39.66               | 38.76           | 37.43           | 145.94              | 118.29               | 159.96           | 122.88           | 72.65                | 117.98                | 134.68            | 132.21            |
| Pluto               | 47.24              | 48.81               | 46.49           |                 | 103.21              | 127.08               | 67.241           |                  | 45.58                | 25.631                | 30.45             |                   |
| Remi                | 32.56              | 35.76               | 36.26           | 35.13           | 180.20              | 228.32               | 182.06           | 182.41           | 96.23                | 220.29                | 111.30            | 91.94             |
| Flash               | 37.17              | 37.28               | 41.08           | 38.33           | 173.78              | 132.89               | 246.37           | 217.88           | 75.06                | 66.80                 | 125.40            | 81.80             |
| Anna                | 33.88              | 37.30               | 38.07           | 35.99           | 204.03              | 216.10               | 198.92           | 220.49           | 105.48               | 75.45                 | 69.63             | 84.53             |
| Major               | 36.00              | 31.49               | 33.65           | 35.27           | 278.58              | 136.99               | 168.32           | 152.89           | 142.52               | 116.75                | 126.13            | 91.73             |

**Table 3.3:** Data values of HRV parameters measured during chamomile treatment

| Chamomile Treatment |                   |                    |                |                |                  |                   |               |               |                  |                   |               |               |
|---------------------|-------------------|--------------------|----------------|----------------|------------------|-------------------|---------------|---------------|------------------|-------------------|---------------|---------------|
|                     | VLF: (%) Baseline | VLF: (%) Chamomile | VLF: (%) Post1 | VLF: (%) Post2 | LF: (%) Baseline | LF: (%) Chamomile | LF: (%) Post1 | LF: (%) Post2 | HF: (%) Baseline | HF: (%) Chamomile | HF: (%) Post1 | HF: (%) Post2 |
| Dewie               | 82.00             | 70.90              | 67.50          | 77.90          | 13.80            | 21.20             | 18.80         | 20.10         | 4.20             | 7.90              | 13.70         | 1.90          |
| Bianca              | 79.77             | 97.56              | 86.04          | 71.56          | 9.94             | 1.44              | 9.05          | 15.87         | 10.28            | 1.00              | 4.91          | 12.57         |
| Moon                | 68.98             | 61.44              | 66.55          | 61.34          | 21.80            | 17.35             | 12.37         | 10.51         | 9.20             | 21.21             | 21.09         | 28.15         |
| Pluto               | 60.67             | 94.77              | 73.51          |                | 34.14            | 4.62              | 22.38         |               | 5.19             | 0.60              | 4.09          |               |
| Remi                | 70.87             | 44.32              | 82.78          | 88.06          | 24.58            | 40.49             | 13.21         | 10.11         | 4.54             | 15.18             | 4.02          | 1.83          |
| Flash               | 87.93             | 84.57              | 83.49          | 93.41          | 9.37             | 10.40             | 8.35          | 5.07          | 2.69             | 5.03              | 8.16          | 1.52          |
| Anna                | 78.79             | 87.02              | 92.85          | 90.52          | 15.22            | 11.94             | 6.18          | 7.13          | 5.99             | 1.04              | 0.98          | 2.35          |
| Major               | 81.85             | 30.69              | 60.35          | 76.49          | 10.11            | 47.33             | 29.97         | 15.80         | 8.01             | 21.98             | 9.68          | 7.71          |

**Table 3.4:** Data values of HRV parameters measured during chamomile treatment

Tables: 3.5 and 3.6

| Water Treatment |                          |                       |                       |                       |                           |                        |                        |                        |                            |                         |                         |                         |
|-----------------|--------------------------|-----------------------|-----------------------|-----------------------|---------------------------|------------------------|------------------------|------------------------|----------------------------|-------------------------|-------------------------|-------------------------|
|                 | HR:<br>(bpm)<br>Baseline | HR:<br>(bpm)<br>Water | HR:<br>(bpm)<br>Post1 | HR:<br>(bpm)<br>Post2 | SDNN:<br>(ms)<br>Baseline | SDNN:<br>(ms)<br>Water | SDNN:<br>(ms)<br>Post1 | SDNN:<br>(ms)<br>Post2 | RMSSD:<br>(ms)<br>Baseline | RMSSD:<br>(ms)<br>Water | RMSSD:<br>(ms)<br>Post1 | RMSSD:<br>(ms)<br>Post2 |
| Dewie           | 37.16                    | 45.27                 | 48.34                 | 38.03                 | 232.06                    | 228.30                 | 213.46                 | 208.65                 | 159.41                     | 96.48                   | 87.42                   | 107.0                   |
| Bianca          | 35.03                    | 39.01                 | 41.01                 | 38.69                 | 170.90                    | 219.50                 | 210.50                 | 228.20                 | 106.60                     | 174.70                  | 165.20                  | 105.0                   |
| Moon            | 55.30                    | 60.10                 | 56.85                 |                       | 192.60                    | 126.90                 | 236.10                 |                        | 139.10                     | 55.00                   | 193.50                  |                         |
| Pluto           | 49.22                    | 49.13                 | 49.44                 | 48.80                 | 73.68                     | 89.87                  | 63.72                  | 85.64                  | 28.72                      | 32.25                   | 27.29                   | 29.0                    |
| Remi            | 36.67                    | 33.02                 | 36.98                 | 34.48                 | 129.80                    | 249.98                 | 246.38                 | 183.58                 | 94.85                      | 136.26                  | 122.96                  | 104.6                   |
| Flash           | 36.68                    | 37.13                 | 41.64                 | 37.29                 | 93.66                     | 141.49                 | 207.79                 | 125.52                 | 65.87                      | 116.66                  | 74.10                   | 74.9                    |
| Anna            | 33.96                    | 32.86                 | 36.86                 | 41.49                 | 264.25                    | 192.89                 | 283.61                 | 170.22                 | 141.95                     | 85.45                   | 125.36                  | 66.4                    |
| Major           | 45.41                    | 33.76                 | 43.95                 | 32.64                 | 100.40                    | 186.42                 | 178.30                 | 110.58                 | 49.20                      | 169.81                  | 80.00                   | 71.0                    |

**Table 3.5:** Data values of HRV parameters measured during water treatment (chamomile study)

| Water Treatment |                      |                   |                   |                   |                     |                  |                  |                  |                     |                  |                  |                  |
|-----------------|----------------------|-------------------|-------------------|-------------------|---------------------|------------------|------------------|------------------|---------------------|------------------|------------------|------------------|
|                 | VLF: (%)<br>Baseline | VLF: (%)<br>Water | VLF: (%)<br>Post1 | VLF: (%)<br>Post2 | LF: (%)<br>Baseline | LF: (%)<br>Water | LF: (%)<br>Post1 | LF: (%)<br>Post2 | HF: (%)<br>Baseline | HF: (%)<br>Water | HF: (%)<br>Post1 | HF: (%)<br>Post2 |
| Dewie           | 61.90                | 84.75             | 78.02             | 76.24             | 29.63               | 11.50            | 18.32            | 18.37            | 8.48                | 3.74             | 3.66             | 5.39             |
| Bianca          | 79.10                | 56.70             | 57.20             | 75.90             | 18.40               | 30.90            | 27.60            | 14.70            | 2.50                | 12.40            | 15.20            | 9.40             |
| Moon            | 51.20                | 70.70             | 61.30             |                   | 33.60               | 23.80            | 23.30            |                  | 15.20               | 5.50             | 15.40            |                  |
| Pluto           | 79.55                | 75.48             | 70.09             | 83.67             | 17.64               | 22.07            | 25.37            | 12.88            | 2.81                | 2.44             | 4.53             | 3.46             |
| Remi            | 57.40                | 85.90             | 87.75             | 70.25             | 28.80               | 10.03            | 8.60             | 24.59            | 13.79               | 4.07             | 3.65             | 5.16             |
| Flash           | 53.26                | 56.45             | 91.68             | 62.11             | 31.94               | 32.23            | 6.93             | 29.76            | 14.81               | 11.32            | 1.40             | 8.13             |
| Anna            | 88.29                | 79.58             | 82.61             | 89.29             | 8.71                | 18.66            | 13.71            | 8.96             | 3.00                | 1.76             | 3.68             | 1.75             |
| Major           | 87.30                | 27.30             | 87.20             | 83.89             | 8.70                | 58.36            | 8.70             | 12.24            | 4.10                | 14.34            | 4.10             | 3.87             |

**Table 3.6:** Data values of HRV parameters measured during water treatment (chamomile study)

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