

Chapter 5

Materials and Methods

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There were two different studies carried out under this doctoral work as follows:

- i) The changes in cardiovascular responses, baroreceptor sensitivity, and heart rate variability before and immediately following the practice of yoga breathing with intermittent breath holding (YBH) comparing with control session of normal breathing with breath awareness.
- ii) Performance in Stop Signal Task was studied at baseline, and following the practice of YBH and Control sessions.

Methodology of the research has been described under the following sub-headings:

5.1: PARTICIPANTS

5.2: DESIGN

5.3: VARIABLES STUDIED

5.4: INTERVENTION

5.5: DATA EXTRACTION

5.6: DATA ANALYSIS

5.1 PARTICIPANTS

5.1.1 SAMPLE SIZE

The sample size was calculated for the autonomic and cardiovascular variables, based on an effect size obtained from a previous study (Raghuraj & Telles, 2008) based on changes in blood pressure following the practice of *pranayama*. It was calculated using G*Power software (Faul, Erdfelder, Lang, & Buchner, 2007), Version 3.1.9.2, where the Power was 0.95, $\alpha = 0.05$, the effect size (Cohen's *d*) was 1.018, and the recommended sample size resulted in being 27 participants in each group. Allowing a 20-30% dropout rate, it was decided to have 40 participants for each session of YBH and control session. Thirty-Nine participants (17 males + 22 females) completed the study.

For the study in which we assessed the performance in Stop Signal Task, the sample size was calculated based on an effect size obtained from a study assessing the changes in Stop Signal Reaction Time (SSRT) following the practice of *Bhramari pranayama* (Rajesh, Ilavarasu, & Srinivasan, 2014). G*Power software, Version 3.1.9.2, where the Power was 0.95, $\alpha = 0.05$, the effect size (Cohen's *d*) was 0.68 and the recommended sample size resulted to be 30 participants in each group. Allowing a 20-30% dropout rate, it was decided to have 40 participants for each of YBH and control sessions. Thirty-six volunteers (17 males + 19 females) completed all the assessments.

Sl. No	Variable Studied	No. of Subjects	No. of subjects/recording sessions
1	Cardiovascular responses, baroreflex sensitivity and heart	39	39 x 4 = 156

	rate variability		
2	Stop Signal Task	36	36 x 3 = 108

5.1.2 SELECTION OF THE PARTICIPANTS

The participants had a minimum of six months experience in the practice of Yoga, and were regular in their practice. The participants were residential students at Swami Vivekananda Yoga Anusandhana Samsthana, a Deemed University, Bangalore. They had all enrolled for graduate and postgraduate programs in yoga.

5.1.3 INCLUSION CRITERIA

Participants who fulfilled the following conditions were included in the study: (i) Healthy volunteers of both the genders with their age ranging between 18 to 30 years (for the autonomic and cardiovascular variables, the mean age \pm SD was 20.64 ± 1.84 years; and for the performance in stop signal task it was 20.66 ± 1.86 years) (ii) Minimum prior experience of yoga for 6 months (for the autonomic and cardiovascular variables, the mean experience in yoga \pm SD was 33.79 ± 13.04 months; and for the performance in stop signal task it was 34.06 ± 13.40 months). (iii) Participants with normal or corrected vision and hearing. Further details of individual participants are given in Tables 6 and 7.

5.1.4 EXCLUSION CRITERIA

The following criteria were used to exclude the volunteers: (i) The presence of cognitive or neurological disorders, respiratory or cardiac disorders, auditory or visual abnormalities, based on a medical history and routine clinical examination, (ii) intake of medication, which is known to influence cognitive functions, (iii) auditory

deficits assessed by checking the auditory thresholds of each ear separately was excluded, (iv) participants who had difficulty in focusing/concentrating, based on interview, (v) smoking or alcoholism which may have influenced the cognitive functions, (vi) female participants during pregnancy or lactation. Additionally, female participants were assessed avoiding the menstrual phase as the menstrual cycle is known to influence the cognition (Colzato, Hertsig, van den Wildenberg, & Hommel, 2010) and the autonomic nervous system activity (Hirshoren et al., 2002).

5.1.5 ETHICAL CONSIDERATIONS

The participants were explained about the aim and method of the study, and the informed consent was signed by all participants (a sample copy is enclosed in **Appendix-1**). None of them were aware of the hypothesis of the study. The study was approved by the institution's ethics committee (IEC) of Swami Vivekananda Yoga Anusandhana Samsthana, Bangalore. The IEC approval letter is enclosed as **Appendix 2**.

Table 6: The characteristics of participants assessed for the autonomic and cardiovascular variables before and following experimental and control sessions

Sl. No.	Subject Code	Age	Gender	Experience of yoga practice in months
1	MNH	21	Male	48
2	SJY	19	Male	24
3	SVT	22	Male	55
4	SRD	21	Male	48
5	RAV	21	Male	48
6	AMS	18	Male	10
7	LKM	20	Male	34
8	SBS	19	Male	23
9	KST	20	Male	22
10	YSD	22	Male	34
11	BSW	22	Male	48
12	IBO	25	Male	48
13	SLY	23	Male	55
14	VSL	19	Male	48
15	PRB	19	Male	12
16	RJN	24	Male	48
17	STS	20	Male	12
18	NDN	23	Female	40
19	ANJ	19	Female	22
20	SWM	20	Female	40
21	ASH	20	Female	22

22	APR	19	Female	22
23	GYT	18	Female	22
24	CHR	18	Female	22
25	MDN	20	Female	30
26	ASY	19	Female	40
27	NST	21	Female	40
28	NBT	22	Female	42
29	MNS	21	Female	33
30	KRN	24	Female	55
31	PRM	24	Female	40
32	SRG	20	Female	33
33	PNM	18	Female	30
34	SMB	20	Female	30
35	MNK	22	Female	22
36	ARD	22	Female	40
37	MDU	19	Female	12
38	RNI	21	Female	30
39	BHU	21	Female	48
Mean		20.64103		33.78947
St. deviation		1.842298		13.04495

Table 7: The characteristics of participants assessed for the performance in the Stop Signal Task before and following experimental and control sessions

Sl. No.	Subject Code	Age	Gender	Experience of yoga practice in months
1	MNH	21	Male	48
2	SJY	19	Male	24
3	SVT	22	Male	55
4	SRD	21	Male	48
5	RAV	21	Male	48
6	AMS	18	Male	10
7	LKM	20	Male	34
8	SBS	19	Male	23
9	KST	20	Male	22
10	YSD	22	Male	34
11	BSW	22	Male	48
12	IBO	25	Male	48
13	SLY	23	Male	55
14	VSL	19	Male	48
15	PRB	19	Male	12
16	RJN	24	Male	48
17	STS	20	Male	12
18	NDN	23	Female	40
19	ANJ	19	Female	22
20	SWM	20	Female	40
21	ASH	20	Female	22

22	APR	19	Female	22
23	GYT	18	Female	22
24	CHR	18	Female	22
26	MDN	20	Female	30
27	ASY	19	Female	40
28	NST	21	Female	40
29	NBT	22	Female	42
30	MNS	21	Female	33
31	KRN	24	Female	55
32	PRM	24	Female	40
33	SRG	20	Female	33
34	SMB	20	Female	30
35	MDU	19	Female	12
36	RNI	21	Female	30
Mean		20.65714		34.05714
St. deviation		1.862049		13.39654

5.2 DESIGN OF THE STUDY

5.2.1 STRUCTURE OF THE SESSIONS

(i) Autonomic and Cardiovascular variables

Self as control design was adapted and each participant visited the laboratory on two different days. On one of the days the participant practiced the yoga breathing with intermittent breath holding (YBH session), and on another day, normal breathing with breath awareness (control session). The duration of practice in both sessions was for 20 minutes. Recordings were performed for pre (5 minutes) and post (5 minutes) sessions. Assessments were made on two separated days, which were not necessarily on consecutive days, but the time of the day was kept constant.

(ii) Performance in Stop-Signal task

Repeated Measures design was applied for the study, in which the participants visited the laboratory thrice, for the recordings at baseline, and following the YBH and control sessions. The time of the day for assessment was kept constant for all three days.

5.2.2 ORDER OF THE SESSIONS

(i) Autonomic and Cardiovascular variables

Half of the total participants were randomly allocated to YBH session on first day and control session on the next visit to laboratory and the other half had the order reversed. The order of the sessions was allotted randomly using computer generated random numbers. This random allotment was done in order to prevent the influence of being exposed to the laboratory setting for the first time.

(ii) Performance in Stop-Signal task

The order of the three sessions (baseline, YBH and control) was randomized for each subject using random numbers generated using a computer program (www.randomizer.org). This was done to prevent the influence of the possible learning effect in the task.

Figure 2: Schematic representation of the self as control study design used for assessment of Autonomic and Cardiovascular variables

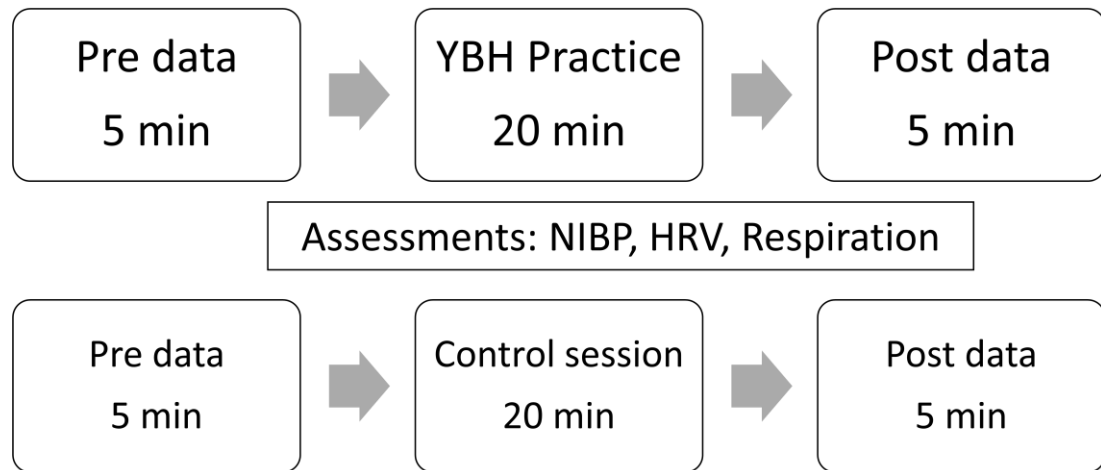
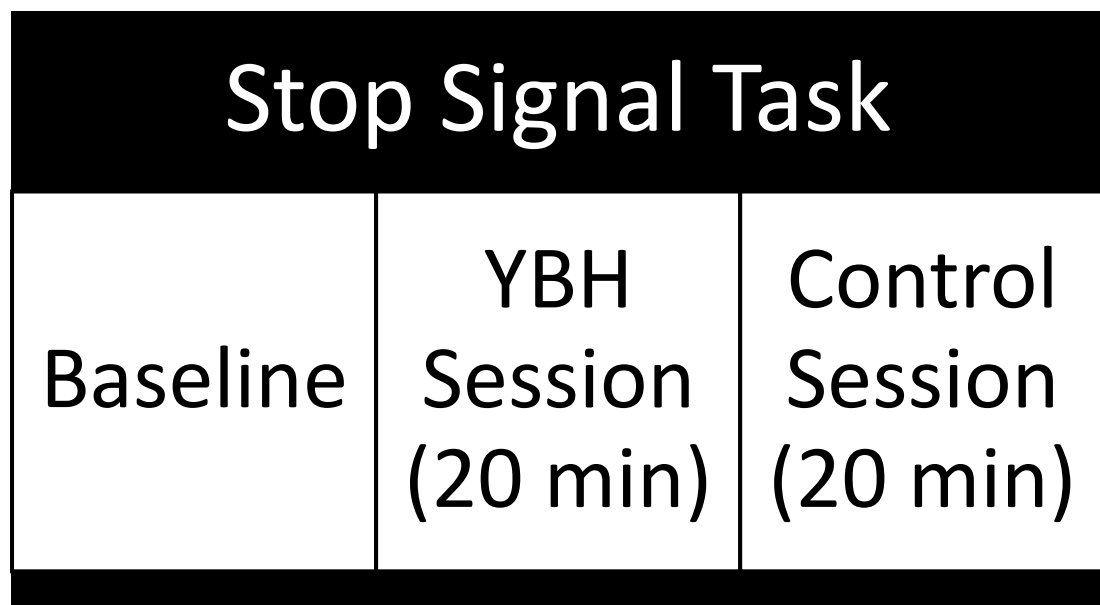


Figure 3: Schematic representation of the repeated measures design adapted for Performance in Stop-Signal task



5.3 VARIABLES STUDIED

The word parameter is described as ‘characteristic of distribution or relationship in the population which are estimated by statistical analysis of a sample of observations’ whereas, the word variable denotes ‘measurement or attribute on which observations are made’ (Altman, Gore, Gardner, & Pocock, 1983). Hence, in this thesis, the term ‘variable’ has been used to describe the assessments studied.

5.3.1 AUTONOMIC AND CARDIOVASCULAR VARIABLES

Heart Rate Variability (HRV) was used as the measure of autonomic activity of the heart. For the assessment of HRV, electrocardiogram (ECG) was recorded using 16-channel human physiology system (PowerLab 16/35, ADInstruments, Australia). The digitized ECG data were analyzed offline to obtain the heart rate variability (HRV) spectrum.

Cardiovascular variables were monitored using Finometer MIDI Continuous Non-Invasive Blood Pressure (NIBP) Systems (Finapres Medical Systems B.V., Netherlands). Bitscope Easy v 2.0 software (Finapres Medical Systems B.V., Netherlands) was used for the recordings of NIBP.

5.3.1. A Rationale for use of the variables

The variables mentioned reflect the activity of the autonomic nervous activity autonomic nervous system. For e.g., the HRV spectrum is believed to be useful indicator of sympathetic activity (reflected by low frequency [LF] band power values) and parasympathetic activity (reflected by high frequency [HF] band power values) (Task Force of The European Society of Cardiology and The North American Electrophysiology, 1996).

5.3.1.B Specifications of PowerLab 16/35

The PowerLab 16/35 is a high-performance data acquisition system suitable for a wide range of research applications that require up to 16 input channels. Typical applications include human and animal physiology. In addition to standard single-ended BNC inputs, the PowerLab 16/35 features 4 differential Pod ports that allow for direct connection of Pod signal conditioners and appropriate transducers.

ECG was recorded using a standard bipolar limb lead II configuration. It was digitized using a 16 bit analog-to-digital converter at a sampling rate of 1 KHz and was analyzed off-line to obtain the HRV spectrum.

5.3.1.C Specifications of Finapres Medical System

Finapres systems are built on combinations of four technologies –

- (i) The volume clamp with physioal technology is the core technology providing accurate finger pressure measurements using the finger cuff (Guelen et al., 2003).
- (ii) The brachial arterial reconstruction technology translates the finger pressure into the commonly used brachial arterial blood pressure (Imholz, Wieling, Langewouters, & van Montfrans, 1991).
- (iii) The Return To Flow (RTF) technology is a technique to calibrate the brachial pressure derived from the finger pressure (Schutte, Huisman, van Rooyen, Malan, & Schutte, 2004).
- (iv) The Modelflow technology is used to derive hemodynamic parameters from pressure data, waveforms and patient data (Jansen et al., 2001).

The accuracy of NIBP by Fianpress Medical Systems has been standardized through comparable experiments with Intra-arterial blood pressure measurements (Imholz et al., 1991; Porter, O'Brien, Kiefert, & Knuppel, 1991).

5.3.1. D Recording Condition

The recording room in the research laboratory was sound attenuated and air-conditioned in order to avoid thermal, visual or auditory disturbance. The temperature of the recording room was maintained at 25 ± 1 °C. The relative humidity during the time of the study was on average 52%. During both practice and assessments, the participants were seated comfortably, keeping the spine erect on a soft chair with backrest.

5.3.1. E Lead/Cuff positions

The ECG was acquired using limb Lead II system, i.e., the electrodes were placed on the right arm and left leg with reference electrode placed on the right leg (Ashley & Niebauer, 2004). Respiration was recorded using a volumetric pressure transducer fixed around the trunk about 8 cm below the lower costal margin while the participants sat erect. A standard finger cuff was connected to the left middle finger, in between the inter-phalangeal joints. Brachial correction was made at regular intervals as per the standard operating procedure of the instrument (Figure 4a and 4b). Figure 4c depicts a subject seated in a sound attenuated cabin with NIBP finger cuff, Lead II EKG and Respiration Belt along with the recording system.

5.3.1.F. Recording procedure

Participants were asked to avoid substances containing caffeine for the day preceding and the day of the recording, since caffeine is known to influence cardiovascular functions (Whitsett, Manion, & Christensen, 1984) and autonomic

responses (Zahn & Rapoport, 1987). Where this was unavoidable, the session was taken on another day. The recordings were taken while the participants were seated with their eyes closed. The recordings were done before, and immediately following the YBH and control sessions.

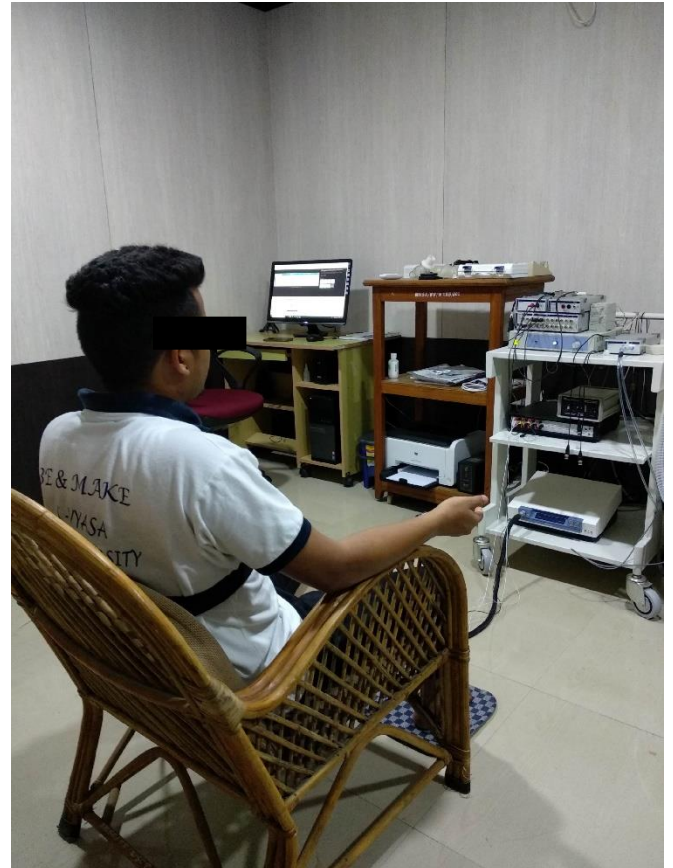
Figure 4 a: The Components of the Finapres Non-Invasive Blood Pressure monitoring system



Figure 4 b: The position of the Finger cuff used for assessment of NIBP



Figure 4C: A subject seated in a sound attenuated cabin with NIBP finger cuff, Lead II EKG and Respiration Belt along with the recording system



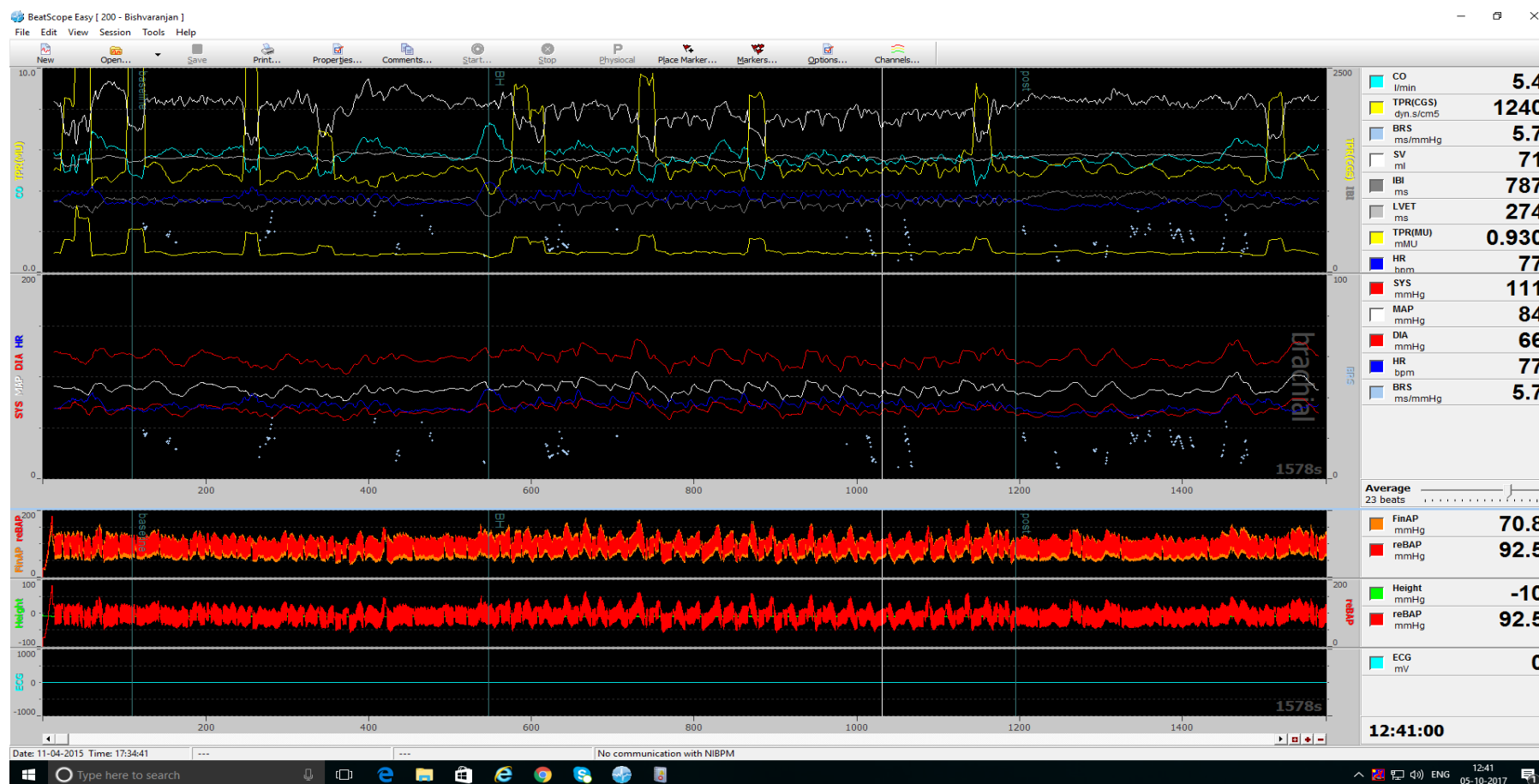


Figure 5: Screen Capture of NIBP recording

5.3.1.G. Variables measured

The following variables were extracted from the 16-channel polygraph.

- (i) The heart rate in beats per minute was calculated by counting the R waves of the QRS complex in the ECG.
- (ii) Frequency domain analysis of HRV: Low frequency (LF) band (0.04–0.15 Hz) and high-frequency (HF) band (0.15–0.5 Hz) and LF:HF ratio.
- (iii) The following components of time domain analysis of HRV revealed the standard deviation of NN intervals (SDNN); the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD); the proportion derived by dividing NN50 by the total number of NN intervals (pNN50).
- (iv) The respiratory rate in cycles per minute (cpm) was calculated by counting the total breath cycles.

The variables studied from the NIBP:

- (i) Systolic blood pressure (SBP) in mmHg
- (ii) Diastolic blood pressure (DBP) in mmHg
- (iii) Mean arterial pressure (MAP) in mmHg
- (iv) Stroke volume (SV) in ml
- (v) Cardiac output (CO) in liters/min
- (vi) The Total Peripheral resistance (TPR)
- (vii) Baroreflex Sensitivity (BRS) in ms/mmHg



Figure 6: Screen Capture of HRV and Respiration Recording

5.3.2 PERFORMANCE IN STOP SIGNAL TASK

5.3.2.A Rationale for use of the variables

Response inhibition is one of the most important aspects of cognition. It is defined as the ability to inhibit unwanted responses according to change in the environment (Logan, Cowan, & Davis, 1984). The Stop Signal Task (SST) has proved to be a useful tool for the study of response inhibition in cognitive psychology, cognitive neuroscience and psychopathology (Verbruggen & Logan, 2008).

5.3.2.B Specifications of the Stop Signal Task

The Stop Signal task is based on the horse-race model where response execution races with the inhibitory process to determine whether a response is inhibited (Band, van der Molen, & Logan, 2003; Verbruggen, Logan, & Stevens, 2008). The primary task is to perform a two-choice task in which subjects had to react as quickly and accurately as possible to discriminate between a left and a right arrow, presented at the center of the computer screen, in white, on a black background. The subject was supposed to respond with the alphabets 'D' (for left arrow) and 'K' (for right arrow) on a keyboard with the left and right index fingers, respectively. On no-signal trials (go task), only the primary task stimulus is presented. On stop-signal trials (Stop Task), an auditory 'stop signal' beep is presented at a variable delay (stop signal delay, SSD) following the go stimulus. Subjects were instructed to inhibit their responses on the trials with a stop signal beep. Tasks were presented randomly: Go task (75%) and stop task (25%). SSD is initially set at 250 ms and is adjusted continuously with dynamically tracking procedure, dependent

upon the performance of the participant. Successful inhibitions resulted in an increase of the SSD by 50 ms, whereas failed inhibitions resulted in a reduction of the SSD by 50 ms. This procedure ensured that on an average each participant in each session had a probability of successful inhibition approaching 50% (Verbruggen et al., 2008). A schematic representation of the stop signal task has been illustrated in Figure 7 based on the work of Logan & Cowan (Logan et al., 1984). A total of 392 trials were presented, divided over six blocks of 64 trials, lasting 3 min each. Subjects waited for 10 s between blocks before they start the next block.

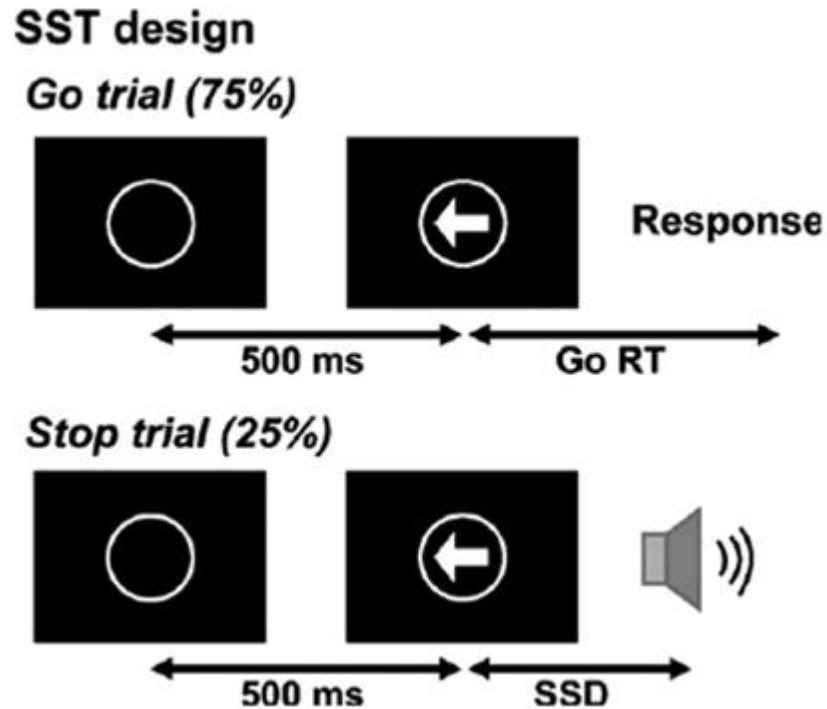


Figure 7: Schematic representation of the stop-signal paradigm. The stop-signal task consists of go and stop-signal trials. A circle is presented for 500 ms, followed by a presentation of an arrow pointing either left or right. Participants are instructed to respond as fast as possible by pressing a left or right button, depending on the direction of the arrow. In the stop trials, an auditory stop signal occurs after the presentation of the arrow, and on these trials, participants must try to withhold their responses. The latency to the sound (the stop signal delay) varies dynamically throughout the study to produce the stop-signal delay 50, where participants can inhibit approximately 50% of their responses. The stop-signal reaction time is calculated as the median go reaction time minus the stop-signal delay 50, according to the race model. Image Courtesy (Madsen et al., 2010)

5.3.1.C. Variables measured

The primary outcome measure was Stop Signal Reaction Time (SSRT), an estimate of the subject's capacity for inhibiting the unwanted motor responses. SSRT was calculated by subtracting mean stop signal delay from mean RT to go stimuli (go RT). Additional measures of interest are the probability of responding on stop signal trials, $p(r/s)$ and Go RT. Figure 8 illustrates the major outcomes of the stop signal paradigm based on the computer program (Verbruggen et al., 2008).

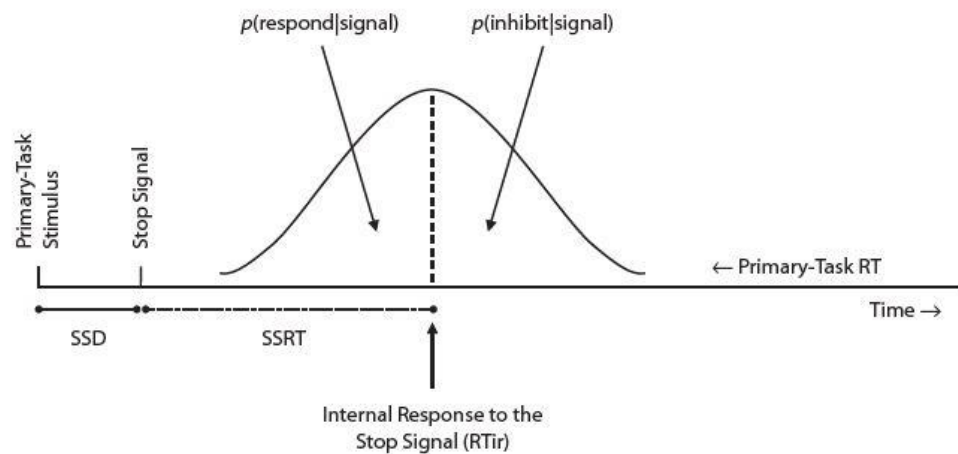


Figure 8: Illustration of the probabilities of responding on stop-signal trials based on the horserace model (Logan and Cowan, 1984), given the distribution of no-signal reaction times (primary task reaction time), the stop-signal delay, and the stop-signal reaction time. Probability of responding on stop-signal trials is represented by the area under the curve to the left of the dashed line (Verbruggen et al., 2008).

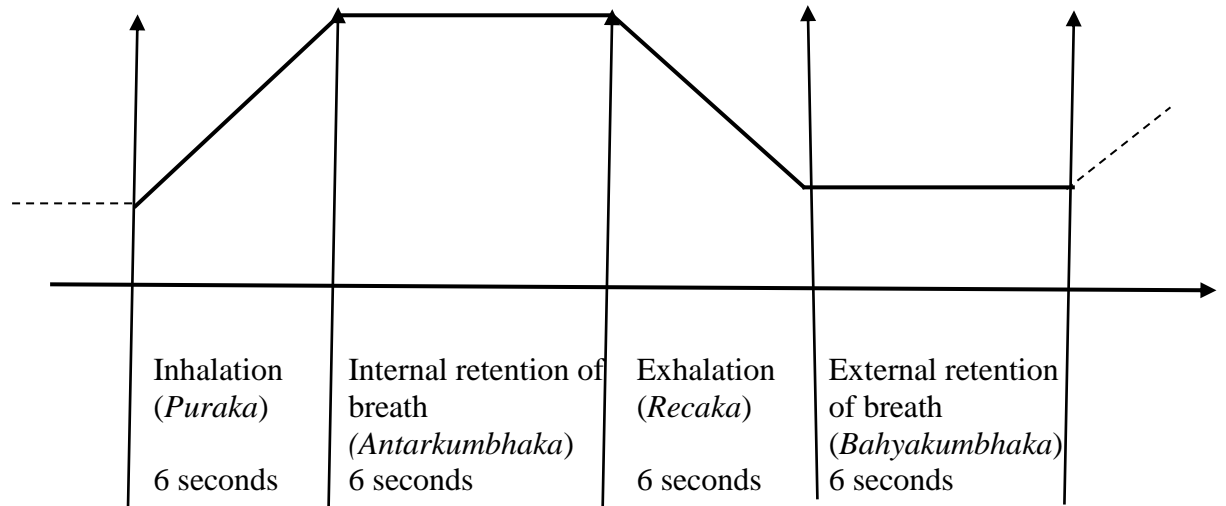
5.4 INTERVENTION

All the participants included in the study were trained in the breathing practice assessed in the present study for 20 min/day, 6 days a week, for 8 weeks prior to the assessment. This 8 weeks of supervised training was conducted to ensure uniformity of breathing practices amongst all the participants.

5.4.1 YOGA BREATHING WITH INTERMITTENT BREATH HOLDING (YBH)

The YBH session included the regulated yogic breathing for 20 minutes incorporating phases of inhalation (*puraka*), internal retention of breath (*antarkumbhaka*), exhalation (*recaka*) and external retention of breath (*bahyakumbhaka*) in a ratio of 1:1:1:1 for 6 seconds each. The classic yoga texts suggest breath retention in varying ratios. The ratio for the intervention was chosen since it is considered ideal for subjects who are naïve to the practice of breath retention. The intervention was derived from a classical training methodology of pranayama suggested in the ancient text of Yoga (Saraswati, 2002). The intervals of 6 seconds were decided based on a previous study which used the similar duration of phases of breath retention along with *Nadisuddhi Pranayama* (Turankar et al., 2013). The duration of 6 seconds was ensured through verbal cues in a pre-recorded audio track. The schematic representation of intervention is done in figure 9.

Figure 9: Schematic representation of one cycle of the practice of yoga breathing with intermittent breath holding.



5.4.2 CONTROL SESSION

During the control session, the participants were seated erect, performing normal breathing with breath awareness for the same duration of 20 min in the same test environment, including the audible cues.

5.4.3 ADVERSE EVENTS

There were no adverse events reported during either the training of participants in YBH or during the assessment sessions.

5.5 DATA EXTRACTION

5.5.1 DATA FROM THE POWERLAB 16/35

The following data were extracted from the 16-channel polygraph. The heart rate in beats per minute was calculated by counting the R waves of the QRS complex in the ECG. Frequency and time domain analysis of HRV data were carried out using Lab Chart 8 (AD instruments, Australia) program, which uses Lomb-Scargle Periodogram algorithm.

5.5.1.A. Frequency Domain Analysis

The energy in the HRV series in the following specific frequency bands were studied viz., Low frequency (LF) band (0.04–0.15 Hz) and high-frequency (HF) band (0.15–0.5 Hz). According to guidelines, LF and HF band values were expressed as normalized units. The LF/HF ratio was also calculated.

5.5.1.B. Time Domain Analysis

The following components of time domain HRV were analyzed: (i) SDNN (the standard deviation of NN intervals), (ii) the square root of the mean of the sum of the squares of differences between adjacent NN intervals (RMSSD), (iii) the proportion derived by dividing NN50 by the total number of NN intervals (pNN50).

5.5.1.C. Respiratory rate

The respiratory rate in cycles per minute (cpm) was calculated by counting the total breath cycles.

5.5.2: DATA FROM THE FINAPRES MEDICAL SYSTEM

Brachial artery systolic (SBP) and diastolic pressures (DBP) were extrapolated from finger arterial pressure through the use of a height correction unit and waveform filtering and level correction methods. Mean arterial pressure (MAP), SBP and DBP were expressed in mmHg. The computed measurements of Stroke volume (SV), cardiac output (CO) from the arterial BP and HR has been found reliable when compared to Modelflow-derived CO (Hill, Iii, & Thayer, 2012). The Total Peripheral resistance (TPR) estimation from the computed CO was also found to be valid (Hill, Iii, & Thayer, 2013). Another variable of interest, Baroreflex Sensitivity (BRS) was estimated from the spontaneous HRV and BP variability (BPV) measured by the Finapres method (Swenne, 2013).

5.5.3 DATA FROM THE STOP SIGNAL TASK

The primary outcome measure was Stop Signal Reaction Time (SSRT), an estimate of the participant's capacity for inhibiting the unwanted motor responses. SSRT was calculated by subtracting mean SSD from mean RT to go stimuli (go RT). Additional measures of interest are the probability of responding on stop-signal trials (p [r/s]) and go RT, extrapolated from the data collected using the computer program INQUISIT 4.0.

5.6 DATA ANALYSIS

The raw data obtained for each subject in each recording session were tabulated separately. The group mean values \pm standard deviation were calculated for all the variables. Statistical analysis was done using statistical package R version 3.2.4 (www.r-project.org) in the following steps.

5.6.1: AUTONOMIC AND CARDIOVASCULAR VARIABLES

- Data were tested for normality by the Kolmogorov-Smirnov test.
- Since the same individuals were assessed in repeat sessions on separate days (ie, YBH and Control), repeated measures analyses of variance (ANOVAs) were performed with two ‘within subjects’ factors, that is factor 1: sessions; YBH and Control and factor 2: states; “pre,” and “Post.”
- This was followed by *post hoc* analyses with Bonferroni adjustment for multiple comparisons between the mean post-state values, and all comparisons were made with the respective pre-state.

5.6.2: PERFORMANCE IN STOP SIGNAL TASK

- Data were tested for normality by the Kolmogorov-Smirnov test.
- Since the same individuals were assessed in repeat sessions on separate days (i.e., baseline, YBH and control), repeated measures analysis of variance was used (ANOVA). Repeated measures analysis of variance (ANOVA) was performed with one 'within-subjects' factors, i.e., Sessions; baseline, YBH and control