Chapter 4

M E T H O D S
This thesis deals with the changes in (i) autonomic and respiratory variables and (ii) middle latency auditory evoked potentials (MLAEPs) in 21 volunteers in 5 sessions each.

4.1 SUBJECTS

4.1.1 Sample size.

The sample size was calculated based on an effect size of previous study of changes in yoga breathing (Telles, Nagarathna & Nagendra, 1994). It was calculated using G-Power software, University of Duesseldorf, Germany; http://www.psycho.uniduesseldorfd.de/aap/projects/gpower. The α level was 0.05, power = 0.95, and the recommended sample size was 16, where δ = 4.0000, Critical t (14) = 2.1448 and the actual power = 0.9602.

4.1.2 Criteria for selection

4.1.2.A Inclusion criteria. (i) The subjects were all healthy on routine medical examination. (ii) Male subjects were selected as MLAEPs (Yadav, Tandon & Vaney, 2002) and autonomic variables (Yildirim, Kabakci, Akgul, Tokgozoglu & Oto, 2002) have been shown to vary with the phases of the menstrual cycles in females. (iii) All subjects had previous experience of the yoga breathing practices ranging from 3 to 48 months. They were regular in their practice and the assessment of their practice was based on the opinion of the yoga instructors in the yoga center. (iv) The subjects were all co-operative and their signed informed consent was obtained (a sample is provided in the Appendix -1).

4.1.2.B Exclusion criteria. (i) Subjects who had abnormalities of the nasal cavities such as nasal septal deviation, nasal polyps, (ii) those who were taking medication
which could influence autonomic function e.g., phenylpropanolamine as a common cold remedy (Lake, Chernow, Zaloga, Labow, Quirk & Hedges, 1988) (iii) upper respiratory tract infection which could cause nasal blockage and (iv) any hearing deficit.

4.1.3 Source. The subjects were students at the yoga center.

4.1.4 Details of the subjects. The subjects all were within the age range of 18 to 45 years (group average age ± SD, 27.48 ± 6.28). Further details of each subject such as their occupation, duration of experience of yoga breathing, daily practice of yoga breathing were noted and are given in Table 4.1.4.

**TABLE 4.1.4**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name Code</th>
<th>Age (years)</th>
<th>Occupation</th>
<th>Duration of experience of yoga breathing (months)</th>
<th>Daily practice of yoga breathing (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>APR</td>
<td>18</td>
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<td>7</td>
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<td>20</td>
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<td>25</td>
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<td>KSH</td>
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<td>45</td>
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<td>student</td>
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<td>24</td>
<td>10</td>
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<tr>
<td>21</td>
<td>VIJ</td>
<td>45</td>
<td>student</td>
<td>5</td>
<td>30</td>
</tr>
</tbody>
</table>

Group mean ± SD: 27.48 ± 6.28

Duration of experience of yoga breathing: 14.62 ± 10.67

Daily practice of yoga breathing: 23.33 ± 13.45
4.2 DESIGN OF THE STUDY

4.2.1 Number of sessions. Each subject was assessed in 5 sessions recorded at the same time of the day. The 5 sessions were sūryānuloma viloma (SAV), candrānuloma viloma (CAV), nādiśuddhi (NDS), breath awareness (BAW) and control (CTL).

4.2.2 Order of sessions. For the 21 subjects the order of the sessions was varied so as to reduce the 'order effect'. For e.g., if subject 1 was studied in 5 sessions in the order SAV, CAV, NDS, BAW, and CTL, subject 2 would be studied in the order CAV, NDS, BAW, CTL, and SAV. This has been detailed by the sample (for 5 subjects) given below.

<table>
<thead>
<tr>
<th></th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>SAV</td>
<td>CAV</td>
<td>NDS</td>
<td>BAW</td>
<td>CTL</td>
</tr>
<tr>
<td>Day 2</td>
<td>CAV</td>
<td>NDS</td>
<td>BAW</td>
<td>CTL</td>
<td>SAV</td>
</tr>
<tr>
<td>Day 3</td>
<td>NDS</td>
<td>BAW</td>
<td>CTL</td>
<td>SAV</td>
<td>CAV</td>
</tr>
<tr>
<td>Day 4</td>
<td>BAW</td>
<td>CTL</td>
<td>SAV</td>
<td>CAV</td>
<td>NDS</td>
</tr>
<tr>
<td>Day 5</td>
<td>CTL</td>
<td>SAV</td>
<td>CAV</td>
<td>NDS</td>
<td>BAW</td>
</tr>
</tbody>
</table>

where S = Subject

4.2.3 Actual design of a session. Each of the 5 sessions lasted for 40 minutes. This consisted of 3 periods namely 'pre' (5 min), test (30 min) and 'post' (5 min). The test period consisted of 30 minutes where the subject practices the respective breathing practice. The initial 10 minutes of the test period was considered as 'adjustment period' where the recordings were not obtained. This was essential as the subject required to establish well in the practice. In this The 30 minute test period differed for 5 sessions so that during SAV there was right nostril breathing, during CAV there was left nostril
breathing, during NDS there was alternate nostril breathing, during BAW there was
breath awareness and during CTL there was random thinking without concentrating on
breathing. During all test periods subjects kept their eyes closed. During the 'pre' and
'post' periods of all sessions subjects were asked to keep their eyes closed and were not
given specific instructions, hence this was similar to the random thinking phase of the
test session. The design has been illustrated in Figure 4.2.3.

Figure 4.2.3:  
DESIGN OF RECORDING SESSION

<table>
<thead>
<tr>
<th>Session</th>
<th>States</th>
<th>Duration</th>
<th>Practice</th>
<th>Post</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAV</td>
<td>Pre</td>
<td>5 min</td>
<td>During practice (20 min)</td>
<td>Post 5 min</td>
<td>30 min (SAV practice)</td>
</tr>
<tr>
<td>CAV</td>
<td>Pre</td>
<td>5 min</td>
<td>During practice (20 min)</td>
<td>Post 5 min</td>
<td>30 min (CAV practice)</td>
</tr>
<tr>
<td>NDS</td>
<td>Pre</td>
<td>5 min</td>
<td>During practice (20 min)</td>
<td>Post 5 min</td>
<td>30 min (NDS practice)</td>
</tr>
<tr>
<td>BAW</td>
<td>Pre</td>
<td>5 min</td>
<td>During practice (20 min)</td>
<td>Post 5 min</td>
<td>30 min (breath awareness)</td>
</tr>
<tr>
<td>CTL</td>
<td>Pre</td>
<td>5 min</td>
<td>During practice (20 min)</td>
<td>Post 5 min</td>
<td>30 min (random thinking)</td>
</tr>
</tbody>
</table>

SAV = śūryānuloma viloma  
CAV = candrañuloma viloma  
NDS = nādiśuddhi  

BAW = breath awareness  
CTL = control (random thinking)
Each subject had 10 recording sessions. There were 5 sessions of different practices for recording autonomic and respiratory variables and 5 sessions for recording middle latency auditory evoked potentials. Hence for 21 subjects there 210 recording sessions.

4.2.4 Familiarization of the subject with the laboratory environment. The subjects were told that the study was intended to understand the physiological effects of the yoga breathing practices. The methods were described and signed informed consent was obtained.

4.2.5 Condition of recordings. The subjects were seated in an air conditioned sound attenuated cabin with dim lighting. The temperature was maintained at 24 ± 1°C. The recording leads led out of the cabin and were connected to the four channel polygraph equipment (Medicaid, Chandigarh, India) and monitored on a closed circuit TV. Instructions were given through two way intercom so that the subjects remained relatively undisturbed during the session.

4.3 VARIABLES STUDIED

The word variable has been used to denote ‘measurement or attribute on which observations are made’ (Altman, Gore, Gardner, & Pocock, 1983), hence in the present thesis the assessments measures have been described as variables.

4.3.1 Autonomic and respiratory variables.

4.3.1.1 Rationale for studying autonomic and respiratory variables.

In the present study the autonomic variables which were measured were the skin conductance, the finger plethysmogram amplitude, the heart rate, heart rate variability (HRV) spectrum and blood pressure (systolic, diastolic and mean pressure). Some
variables mentioned clearly reflect activity in either sympathetic or parasympathetic subdivisions of the autonomic nervous system. For e.g., an increase in skin conductance would reflect increased sudomotor sympathetic activity while a decrease in finger plethysmogram amplitude reflect an increase in sympathetic vasomotor activity (Appenzeller, 1982) 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 Society of Pacing and Electrophysiology, 1996). The regulation of respiration is complex and depends on factors such as blood gas levels, blood pH as well as psychological effects (Ax, 1953).

4.3.1.B Skin Conductance Level (SCL). Two metal plates fitted with velcro tape were in contact with the volar pads of the index and middle fingers of the left hand. A low level DC amplifier was used as one of four channel polygraph (Medicaid, Chandigarh, India). A current of 0.5 V was passed through the applied electrodes. A thin film of electrolyte gel (Electrode Gel, Medicaid Systems, Chandigarh, India) was put on the metal plates. See Figures 4.3.1 (i) and 4.3.1 (ii)

4.3.1.C Respirogram. The respiration was recorded using a stethograph connected to AC amplifier of the polygraph and with sensitivity set as required. The stethograph was fixed around the trunk about 8 cm below the lower costal margin as the subject sat erect (Telles, Nagarathna & Nagendra, 1996). See Figures 4.3.1 (i) and 4.3.1 (ii)

4.3.1.D Finger Plethysmogram. Finger plethysmogram was recorded using photoelectric transducer kept at the base of the nail bed of the left thumb. The
transducer was connected to an AC amplifier of the polygraph and sensitivity was set as required. See Figures 4.3.1 (i) and 4.3.1 (ii)

4.3.1.E Electrocardiogram (EKG). The electrocardiogram was recorded placing electrodes 1 cm below the mid-clavicular point on either side of the chest. This was to simulate the standard limb lead I configuration since connecting the leads on the left arm and right arm (limb lead I position) would be expected to result in artifact as the subjects used their right hand to manipulate the nostrils. See Figures 4.3.1 (i) and 4.3.1 (ii)

4.3.1.F Heart Rate Variability (HRV). The EKG was digitized using a 12 bit analog-to-digital converter (ADC) at a sampling rate of 500 Hz and stored on the hard disk of a PC (Pentium) for analysis. The R waves were detected to obtain a point event series of successive R-R intervals, from which the beat to beat heart series was computed. The data recorded were visually inspected off-line and only noise free data were included for analysis. In this study the HRV was recorded on 21 subjects as for other variables, however due to certain technical errors it was not possible to analyze the HRV data in some of the 'during' periods for all subjects and especially for one subject (subject code: V17) the data were unavailable hence the data of 20 subjects are presented.

4.3.1.G Blood Pressure. The blood pressure was recorded with a standard mercury sphygmomanometer, auscultating over the right brachial artery. The diastolic pressure was noted as the reading at which the Korotkoff sounds appear muffled (Telles, Nagarathna & Nagendra, 1996). The mean pressure was also noted. This is the average pressure throughout the cardiac cycle. The mean pressure is obtained using
mathematical derivation = diastolic pressure + 1/3 X Pulse pressure. Where the pulse pressure is the difference between the systolic and diastolic pressures (Ganong, 1987).  

4.3.1.H *Subjective impressions*. The subjective impression about the quality of practice was assessed on analog scales (0 to 10 cm, where 0 = worst and 10 = very good). Subjects were asked to note each of their practices immediately after the session (a sample is provided in the Appendix-3).
Subject seated while practicing yoga breathing technique in sound attenuated cabin with transducers connected to polygraph to record skin conductance level, EKG, respiration, and finger plethysmogram amplitude.

Figure 4.3.1 (ii): 4 channel polygraph (Medicaid, Chandigarh) to record autonomic and respiratory variables.
4.3.2 Computer averaged middle latency auditory evoked potentials (MLAEPs).

4.3.2.A Rationale for studying middle range of evoked potentials.

MLAEPs were chosen for this study with the premise that the conscious processes of breath awareness actively involved several cortical mechanisms and that corticofugal controls may exert significant alterations in the processing of information at the brainstem and thalamic levels (Steriade & Llinas, 1988; Telles, Joseph, Venkatesh & Desiraju, 1992; Raghuraj & Telles, 2004). There are no studies available in all yoga breathing practices compared with a control as mentioned in this thesis. Hence it was preferred to workout in detail changes in the mesencephalon-diencephalon and primary auditory cortex levels which are the generators for MLAEPs (Liégeois-Chauvel, Musolino, Badier, Marquis & Chauvel, 1994).

4.3.2.B Testing procedure. The subjects were first tested in a sound attenuated cabin and their hearing threshold for each ear was determined separately by giving monaural click stimuli through close fitting ear phones (Amplivox, UK). This served to rule out any hearing deficit. The average hearing threshold (binaural) for the group was noted. See Figures 4.3.2 (i) & 4.3.2 (ii).

4.3.2.C Amplifier settings. The middle latency auditory evoked potentials were recorded using the Nicolet Bravo System (U.S.A). The MLAEPs were computer averaged in 1500 trial sweeps, in the 10-100 ms range. The electroencephalographic (EEG) activity was amplified with a sensitivity of 50μV and filter bandpass 5 to 1000 Hz. The artifact rejection was kept at 90%. There was no pre-stimulus delay.

4.3.2.D Stimulus characteristics. Binaural click stimuli of alternating polarity and 50 μs in duration with a frequency of 5 Hz were used to trigger online averaging of the
EEG. The stimulus intensity was kept at 80 dBnHL which was shown in a previous study to be adequate to evoke the responses without disturbing the subjects' attention to the breathing practices (Telles, Joseph, Venkatesh & Desiraju, 1992; Raghuraj & Telles, 2004).

4.3.2. Electrode positions. The recording sites were prepared using electrode gel (Ten20 Conductive EEG Paste, D.O. Weaver & Co., USA). Ag/AgCl disk electrodes were used for recording. There were three active recording sites C3, C4 and C7 according to the International 10-20 system (Jasper, 1958). The montages were as follows: C3 referenced to the left earlobe (A1), C4 to the right ear lobe (A2), and C7 referenced to the left ear lobe with the ground electrode kept on the forehead (FP2). All electrode impedances were kept below 5 kilohms throughout the session.
Figure 4.3.2 (i): Subject seated while practicing yoga breathing technique in sound attenuated cabin with (1) electrodes at CZ, C3, C4 (active), FPZ (ground), and both ear lobes (as reference) and (2) wearing acoustically shielded ear phones to record middle latency auditory evoked potentials.

Figure 4.3.2 (ii): The Bravo EP system (Nicolet, USA) - 4 channel amplifier and closed circuit TV monitor on the left.
4.4 DATA EXTRACTION

4.4.1 Autonomic and respiratory variables.

4.4.1.1 Skin Conductance Level (SCL). The SCL values were taken at intervals of 30 seconds from the continuously made record. The SCL values were averaged in a five minute block period. See Figure 4.4.1

4.4.1.2 Respiratory Rate. Readings were obtained for every 60 seconds as number of cycles per minute. The readings were averaged for each five minute block period. See Figure 4.4.1

4.4.1.3 Finger Plethysmogram Amplitude. The finger plethysmogram amplitudes were noted every 30 seconds in each five minute block period. The amplitude of the finger plethysmogram were sampled from the peak of pulse wave at 30-second intervals and presented in cm. This was the finger plethysmogram amplitude (Vempati & Telles, 2002). See Figure 4.4.1

4.4.1.4 Heart Rate. The heart rate was obtained by counting the R waves of the QRS complex in successive epochs of 60 seconds and averaged for each 5 minute block period. See Figure 4.4.1

4.4.1.5 Heart Rate Variability (HRV). The HRV power spectrum was obtained using fast Fourier transform analysis (FFT). The energy in the HRV series of the following specific bands was studied, viz. the very low frequency component (0.0-0.05 Hz), low frequency component (0.05-0.15 Hz), and high frequency component (0.15-0.50 Hz). The low frequency and high frequency values were expressed as normalized units, which represent the relative of each power component in proportion to the total power minus VLF component [LF norm = LF / (total power-VLF) x 100; HF norm = HF /
(total power-VLF) x 100] (Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). See Figure 4.4.1.E

Figure 4.4.1: A typical tracing of polygraph record showing skin conductance level, heart rate, respiratory rate, and finger plethysmogram amplitude

Figure 4.4.1.E: A typical tracing of heart rate variability spectrum. The vertical axis gives the power values in BPM²/Hz. The two vertical dotted lines separate the three frequency components, viz., very low frequency (VLF), low frequency (LF) and high frequency (HF). The arrows indicate the highest peak in each range.
4.4.2 Computer averaged middle latency auditory evoked potentials (MLAEPS).

The Na, Pa and Nb waves were studied. These waves were detected in relation to the Pa wave (Morlet, Bertrand, Salord, Boulieu, Pernier & Fischer, 1997), as it appears to be most the prominent, robust and stable component in adults (Kileny, Paccioiretti & Wilson, 1987), as follows: the Na wave was the maximum negative peak preceding the Pa wave, which is a positive component occurring between 25 and 32 ms. The Nb wave was taken as the first maximum negative component immediately following the Pa wave. Computerized quantification of peak amplitudes and peak latencies of these three waves was made with the pre-stimulus baseline as reference. See Figure 4.4.2

Figure 4.4.2: A typical tracing of middle latency auditory evoked potentials
4.5 DATA ANALYSIS

For each subject the average of data (autonomic and evoked potential components) was obtained for each of the phases (pre, during, post) for five types of sessions (SAV, CAV, NDS, BAW and CTL).

Hence for each variable assessed of a single subject there was a single value for pre, four values for ‘during’ and a single value for ‘post’ periods.

These values were tested for variance, and since all cases the data were interval data and values being compared had equal variance, parametric tests were used for analysis.

First, repeated measures ANOVA were performed using SPSS (version 10) with two Within - subjects factors. For each variable (e.g., heart rate) two repeated measure ANOVAs were performed: Within subjects Factor 1 (Sessions: SAV, CAV, NDS, BAW and CTL; 5 levels) and Within subjects Factor 2 (States: pre, during 1, during 2, during 3, during 4, and post, 6 levels).

Subsequently paired t-tests were performed to compare ‘during’ (during 1 to 4) and ‘post’ periods with the respective ‘pre’ period of five sessions.
4.6 PRĀÑAYĀMA TECHNIQUES

The subjects were seated comfortably with the back erect keeping the shoulders relaxed and eyes closed.

Śūryānuloma viloma (SAV) or right nostril yoga breathing involves breathing through the right nostril, while the left nostril is occluded with gentle pressure from the ring and little fingers of the right hand (nāsika mudra in Sanskrit) (Swami Niranjananda Saraswathi, 1994).

Candrānuloma viloma (CAV) or left nostril yoga breathing involves breathing through the left nostril, while the right nostril is occluded with gentle pressure from the thumb of the right hand (nāsika mudra in Sanskrit).

Nadiśuddhi (NDS) or alternate nostril yoga breathing starts with exhalation through both the nostrils, followed by closing the right nostril with the thumb of the right hand and inhaling slowly through the left nostril. After complete inhalation, the left nostril is closed with the little and ring fingers of the right hand followed by opening the right nostril and exhaling through it. This forms one round of NDS (Nagendra, Mohan & Shriram, 1988).

During breath awareness (BAW) the subjects were asked to be aware of their breathing without any manipulation.

In the control (CTL) session the subjects were seated quietly in random thinking and without being aware of breathing.