‘Is Going through Clinical Test a Headache?’
An HRV Study and Descriptive Report of Subjective Experience of Undergoing EEG Testing

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Introduction
In the past few decades, there has been a rapid escalation in use of diagnostic and clinical testing facilities for various diseases and health checkups [1]. Clinical tests may vary from a simple venesection to prolonged procedures like electroencephalography (EEG). This prolonged duration during investigations may produce additional stress over and above the stress of disease.

EEG is routinely being used for epilepsy, seizures [2] and cognitive neuroscience research. The EEG requires placement of many disc electrodes with long flexible lead on the scalp. The prerequisites for good signal acquisition include moderate rubbing, debridement of scalp with scrubbing agent and cleaning with alcohol swab at each site. The procedure is routinely done in a sitting posture and preparation for testing protocol usually takes 30–60 min. Alternatively, the cap system of EEG signal acquisition can be used but it has its own limitations. The common notion is that EEG preparation and procedure is ex-
haustive and tiring but the possibility that it may be perceived as soothing massage needs to be explored. Recently, massage therapy has emerged as one of the alternative treatments [3] and is often considered as a technique of relaxation [4]. Studies involving processes like gentle stretching and moderate cutaneous pressure have shown to decrease tension and create a state of tranquility [5].

The stress and relaxation responses have been associated with discrete changes in autonomic nervous system (ANS), which can be measured by heart rate variability (HRV) [6, 7]. Measuring HRV is a simple, sensitive and non-invasive technique based on beat-to-beat alteration in heart rate [8]. HRV is supposed to be an indicator of the level of stimulation of ANS and dynamic interaction and balance between the sympathetic nervous system and the parasympathetic nervous system [6, 9–11].

HRV has been used to measure changes in sympathovagal activity during massage therapy [12], emotional states (such as stress and anxiety) and pain studies [6, 13–16]. The findings from meta-analysis of several studies have established a link between emotions, such as anxiety and hostility, and a reduced HRV [8]. Therefore, exploring the emotional variables that may affect HRV may be beneficial to the heart and health research [17].

We assessed subjective perception and HRV changes while undergoing EEG. To the best of our knowledge, no such prior study is available in literature.

**Methods**

**Study design:** prospective cohort study.

**Study Population**

We conducted a study on 35 healthy male volunteers who were randomly selected and recruited from an ongoing research study in a cognitive neuroscience laboratory. Only male participants were included because women tend to have different perceptions of relaxation responses, mental stress and stress reactivity during the different phases of menstrual cycle [18]. Participants with history of cardiac diseases, high blood pressure, diabetes or prior treatment for any medical condition or those indulging in substance abuse, smoking and alcoholic were excluded.

We briefed the participants about the study through a subject information sheet after obtaining written informed consent. Participants were requested to report in the cognitive neuroscience lab at 10 a.m., to not eat for 4 h prior to the experiment, refrain from undue body movements, talking and falling asleep and specifically instructed not to voluntarily change their breathing pattern during electrocardiogram (ECG).

Ethical clearance for the study was taken from the institutional ethics committee for the human subjects.

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**Study Protocol**

| Relaxation on chair for 10 min |
| Record ECG from lead-2 for 5 min |
| Placement of EEG electrodes approximately in 30 min |
| Record ECG from lead-2 for 5 min |
| Perception of EEG electrode placement on 3 point likert scale (relaxing, neutral, uneasy) |

**EEG Electrodes Placement Protocol**

The participants were instructed to clean their hair with shampoo without the application of conditioner or oil 1 day prior to the testing. A total of 23 silver-silver chloride (Ag-AgCl) disc-type electrodes of 5–7 mm diameter with long flexible leads (21 scalp electrode and 2 ear electrodes) were placed on the scalp according to the 10–20 international electrode placement system with the help of ‘Elefix’ paste for EEG (supplied by Nihon Kohden). An electrode impedance of less than 5 kΩ at each position was achieved by cleaning scalp with alcohol swab followed by rubbing with skin preparation gel ‘SKINPURE’ (supplied by Nihon Kohden). The whole procedure was performed in a sitting posture and lasted 30 min. EEG electrodes of RMS EEG-19 Superspec system were used.

**ECG Signal Acquisition and HRV Analysis**

ECG signals were acquired using the lead-2 at sample rate of 200/s and gain X 2,000 from power lab data recording system (ADInstruments, Australia). HRV analysis was based on a 5-minute period of ECG signal using LabChart software (ADInstruments, Australia). The heart rates were calculated using the R-waves from artefact-free graph for both stages of recordings. Time domain and frequency domain analysis of HRV of both sessions was done offline.

The parameters used were set as follows: total power frequency (TPF) (0.00–0.40 Hz), low frequency (LF) power (0.04–0.15 Hz) and high frequency (HF) power (0.15–0.40 Hz). The LF/HF ratio was calculated based on the outcome of power spectral analysis. The following HRV parameters were analyzed.

**Time domain parameters:**

- Average heart rate (bpm)
- SD heart rate (bpm)
- SDSD
• RMSSD
• pRR50%
    Frequency domain parameters:
• LF (nu)
• HF (nu)
• LF/HF ratio

Statistical Analysis
The statistical analysis was done using the SPSS software version 20. The distribution of the data was found to be non-Gaussian type; therefore, non-parametric test (Wilcoxon signed-rank test) was applied. HRV comparison of pre- and post-EEG electrode placement was done, and p values <0.05 were considered significant.

Results
The mean age of the participants in the study was 30.15 years. The detailed anthropometric parameters of the participants are given in the table 1.

Self-Assessment Description
Out of the 35 participants, 13 (37%) participants perceived the experience of EEG electrode placement as relaxing. For 11 (31.5%) participants, the experience was neutral. The remaining 11 (31.5%) participants complained of uneasiness.

The participants who perceived the procedure as a gentle massage, soothing in nature and conducive for induction of sleep reported the experience as relaxing in the 3-point Likert scale. The reason for uneasiness was discomfort, tiredness and a feeling of constrain that was due to prolonged sitting in the same posture. The mixed feeling was reported as neutral.

HRV Results
The comparison of before and after 5 min of each fast Fourier transform applied epoch of ECG showed no significant differences in any of the time and frequency domain parameters of the HRV.

The results of subjective perception and HRV analysis are summarized in figure 1 and table 2.

Discussion
The present study was carried to find out the HRV changes and subjective perceptions of undergoing EEG. The subjective experiences of the procedure ranged from relaxing to uneasy. These differences may be because of varied temperament, mood and personality types of the volunteers. Stress response is dependent on the environ-

Table 1. Anthropometric parameters of the study participants (n = 35)

| Parameters     | Mean ± SD    |
|----------------|-------------|
| Age, years     | 30.15±6.30  |
| Height, cm     | 166±25.32   |
| Weight, kg     | 62.64±9.01  |
| BMI, kg/m²     | 22.69±3.19  |

BMI = Body mass index.

Table 2. Parameters during pre- and post-electrode placement

| Parameters during pre- and post-electrode placement | HRV frequency |           |           | p value |
|-----------------------------------------------------|---------------|-----------|-----------|---------|
|                                                     | pre           | post      |           |         |
| LF (nu)                                             | 58.90±19.80   | 62.82±19.28| 0.235     |
| HF (nu)                                             | 40.51±19.16   | 36.25±18.21| 0.172     |
| LF/HF                                               | 2.49±2.98     | 2.68±2.19  | 0.140     |

|                                                     | HRV time domain |           |           | p value |
|-----------------------------------------------------|-----------------|-----------|-----------|---------|
|                                                     | pre             | post      |           |         |
| Average heart rate, bpm                              | 81.08±10.84     | 79.82±9.76| 0.32      |
| SD heart rate, bpm                                   | 5.01±1.83       | 4.46±1.64 | 0.067     |
| SDSD                                                | 34.21±23.96     | 31.81±25.59| 0.067     |
| RMSSD                                               | 34.17±23.93     | 31.77±25.55| 0.07      |
| pRR50%                                              | 13.38±17.21     | 11.11±16.89| 0.08      |

All values are mean ± SD.

Fig. 1. Descriptive self-report of EEG electrode placement experience (n = 35).
Another possibility is the mobilization of different cognitive faculties as the key players in stress appraisal response. The stress response depends not only on the specific type of stressor but it commences after the cognitive appraisal of stimulus as a threat [20].

Scientific studies to assess the effects of massage on the ANS are limited, yet evidences suggest varied findings. An increase in both parasympathetic [21] and sympathetic [22] activities have been reported with massages. Subjective relaxation is a common finding with different types of massage although a physiological relaxation response depends on the type, duration or site of massage. These varied results may be dependent on increased or decreased arousal as indicated by heart rate and EEG activity depending on the type (light, moderate or vibratory) of massage [23].

In the present study, we found no significant differences in time and frequency parameters of HRV. Although HRV parameters are useful and it is a sensitive tool to assess ANS activity during various emotional states, anxiety, therapeutic touch, massage [12] and pain [6, 13–16], the lack of clear cut patterns in HRV may be because of varied reasons. The first possibility is the differences in individual perception of the intervention. The second possibility is varied experiences with temporal progression, with the same participant having experienced 2 opposite extremes of experiences over and over again, which may have cancelled out sympathetic and parasympathetic responses. The procedure of EEG electrodes placement may have evoked relaxation response due to parasympathetic activation resulting from gentle soothing massage and touch or stress response resulting in sympathetic activation due to fatigue, exhaustion and restriction or mixed typed response. Third is that the intervention was gentle and hence failed to activate any physiological autonomic response. Though this is quite unlikely, yet it cannot be ruled out. Another alternative explanation may be that the effect was too small to be measured or the instrumentation system not sensitive enough to detect the effects.

The scenario may also point at the effect being masked by the interplay of unknown variables or the effect of responders in the study population being masked by opposite effects from non-responders. The variations in response of individual may have first evoked sympathetic and later on parasympathetic responses or vice versa and a more complex cyclical response with the temporal progression of the procedure over a period.

A variety of variables ranging from physical to mental have been reported in literature to significantly alter HRV and they can be quantified by average LF/HF ratio. It may range from controlled breathing at 15 breaths per minute to performing mental tasks [24, 25]. Therefore, during the study, all confounding factors like physical, mental and others that may affect HRV should be taken into consideration through randomization [25]. Still stringently randomized controlled studies may get confounded by participant’s mental activity with the temporal course of the study and mental activity is deemed as the factor having an utmost effect on the HRV. Controlling mental activity, however, is nearly impossible, with the possible exception of study during sleep [25], which was not feasible in our experimental settings.

In summary, undergoing EEG has varied experience and no significant change in HRV may be due to the mix heterogeneous autonomic response, different individual personality types (sympathetic type, parasympathetic type and non-responder) and complex mental states originating with temporal progression of task. However, the possibility may be that no stress is generated during clinical test.

The results of our study may be specific to our participants and their perceptions of intervention in our laboratory settings.

**Authorship Contributions**

R.K.: data collection, drafting of manuscript, statistical analysis; Y.S.: data collection, drafting, interpretation of result; A.G.: critical revision of draft and valuable input; P.P.: design of study.

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**Disclosure Statement**

None.

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