Attentional focus refers to the mental inhibitory capacity an individual draws on when performing a skilled motor task. How an individual manages his mental resources will have a strong effect on motor performance. Often in sports, poor performance is due to errors of attention or the inability of athletes to focus on the task at hand. How an athlete responds to challenge can dictate their ability to be successful on the field. Electroencephalography (EEG), a measure of brain activity, and heart rate variability (HRV), an index of autonomic (i.e., parasympathetic) activity, have been used as measures of attentional focus and, as such, can provide important insights into critical neural processes associated with attention and sport performance. **PURPOSE:** To index an individual’s psychophysiological biomarkers (EEG and HRV) and validate how they would change as task difficulty increases. **METHODS:** Thirty-five healthy participants (ages ranged from 18-40 years) were recruited from the University of Maryland, College Park. Thirty-one (n = 31) of the participants provided usable data for the analysis. They performed a visuo-motor task in a Snake® game with two levels of challenge (Easy vs Hard) that lasted five-minutes. **RESULTS:** EEG data analysis was performed using a 2 x 2 x 5 (Condition x Cerebral Hemisphere x Brain Region) ANOVA. The results of the experiment revealed an elevation in neural effort, as indexed by regional theta band power (4-7 Hz), which suggests an increase in cerebral cortical activation with increased task difficulty (F (1, 30) = 27.15; p < 0.005). HRV data analysis was calculated using a repeated measure ANOVA and the results revealed a withdrawal of parasympathetic influence, and an increase in sympathetic dominance with increased challenge ((i.e., increased high frequency(0.15 - 0.4 Hz) HRV (F (1,30) = 9.074; p = 0.005)), low-frequency HRV (0.04 - 0.15 Hz) (F (1,30) = 9.074; p = 0.005), and low-frequency to high-frequency ratio (F (1,30) = 5.185; p = 0.3). **CONCLUSIONS:** The results of this experiment support the validity of EEG and HRV as objective measures of attention demand and, furthermore, that withdrawal of parasympathetic activity is associated with increased mental workload as measured by EEG theta power.