

Research Article



The Linear and Nonlinear Indices of Electroencephalography Change in the Stroop Color and Word Test

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ABSTRACT

Introduction: This study evaluated the brain activity based on the linear and nonlinear features of surface electroencephalography (EEG) in the Stroop Color and Word Test (SCWT) and the effect of learning in the test response and related EEG features.

Materials and Methods: A total of 21 women and 19 men with physical and mental health participated in this study. Four stages of this SCWT, consistently in the first and second stages and inconsistently in the third and fourth stages, were taken twice by the participants with a 10-min interval. Besides, EEG recording was simultaneously taken for 1 minute at each stage.

Results: The number of correct responses in the inconsistent stages was lower than that in the consistent stages, while the delay of correct responses was more in the consistent stages. EEG features showed that the relative power band of alpha 1 (8-10 Hz) frequency reduced during the test compared to the resting state. In contrast, the gamma 2 (40-50 Hz) frequency band showed a significant increase. There was no significant difference between various stages of the test and between two repetitions in the test indices and EEG features.

Conclusion: Compared to the resting state, the relative power of alpha 1 and gamma 2 frequency bands changed during SCWT without considering the stage of the test.

1. Introduction

Paying attention is one of the most critical executive functions of the brain and the basis of other cognitive processes. Attention is a cognitive process defined as the

selective focus on one part of the environment while other parts are ignored [1]. Stroop Color and Word Test (SCWT) is widely known to assess selective attention, in which different variables manipulate the correct selection. The word and its color are sometimes consistent or inconsistent based on various stages of the test.

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Changing the confounding factors, the delay of responses, and their correctness and incorrectness will change. Therefore, the result becomes a criterion for measuring the participants' selective attention in the health subjects [2]. Soleimani et al. determined that the reaction time in the Stroop test is significantly related to creativity [3]. The SCWT, as one of the most widely used tests assessing selective attention, is a laboratory model to test the function of the prefrontal cortex and some other parts of the brain, especially the anterior cingulate cortex [4]. Some studies have shown brain areas in attention based on Functional Magnetic Resonance Imaging (fMRI). These areas include the posterior parietal cortex, which provides a representation of the sensory map of the space to which attention is directed; the anterior cingulate cortex, which plays a motivating and predictive role and determines what should be considered and what should be ignored; the frontal cortex, which coordinates movement programs for attention-related activities such as staring at specific areas of the visual field, focus in the visual field, or reaching for an object; network structures, which create different levels of alertness; the upper annular ridges of the midbrain, which divert attention to a new location and finally; and the thalamus, which is used to draw attention and focus on a new location [5, 6]. Notably, the SCWT activates these areas of the brain. On the other hand, some studies determined that SCWT activates the stress system, enhancing the mind-wondering and stress [7, 8].

A critical tool for measuring brain function, especially at the cortical level, is Electroencephalography (EEG). This tool can reflect the brain's outcome behavior during selective attention by reflecting instantaneous events (in ms) of the brain on surface electrodes. EEG recording, although less spatially sensitive, has a better temporal sensitivity compared to the fMRI records. As the number of EEG electrodes increases, the smaller sets of neurons are covered by each electrode. Therefore, it increases spatial accuracy as well. Besides, computational methods can reduce volume conductor error in EEG recording [9]. Researchers in 2017 indicated that during the SCWT, the power of theta, alpha, and gamma frequency bands in the prefrontal cortex significantly increase compared to the resting state [10]. In 2018, Shriram et al. showed that the brain connections would change following the consistent/inconsistent SCWT, while more changes would occur in the theta and gamma bands. There was a strong positive correlation between frontal EEG sub-bands and the consistent SCWT. There was also a significant positive correlation in the frontal and temporo-frontal regions [11]. Atchley et al. showed that in cases where individuals had errors in the SCWT, theta and alpha bands' activity significantly decreased [7].

The question is whether there is a difference between the EEG signal indices in various stages of the SCWT, from simple to complex, which requires more attention. In another study, Stinch et al. suggested that a frequency band between 13 and 20 Hz is sensitive to the difference between the consistent and inconsistent SCWT. In terms of inconsistency, high connectivity is observed between the left frontal and left peritoneal regions. Less delay in response was accompanied by greater connectivity of the right frontoparietal area [12]. In 2015, Hugh et al. concluded that while SCWT became harder in each stage, the individual became more stressed. On 9 healthy young people, it has been revealed that the support vector machine classifier is trained by EEG signal features such as relative power band and nonlinear indices such as fractal dimensionality. They showed a prediction accuracy of over 85% to separate stages of the SCWT according to the intensity of stress perception. In this study, the criteria were the level of perceived stress, not merely the stage of the SCWT [13].

The purpose of this study was to investigate whether linear and nonlinear indices of the EEG signal can distinguish between various stages of the SCWT, from easy to complex, by statistical analysis and artificial neural network classification. The test learning in the SCWT was also assessed after doing it twice with 10-minute intervals.

2. Materials and Methods

A total of 21 right-handed women and 19 right-handed men participated in this study in the Baqiyatallah University of Medical Science in 2018. They were physically and mentally healthy. The inclusion criteria were no history of head and spine surgery, no drug and psychedelic use, no systemic and cardiovascular disease, and no regular or professional exercise. People were asked not to attend the test if they were suffering from acute stress. After being informed of the study process, the participants signed the informed consent form approved by Baqiyatallah University of Medical Sciences. All participants completed the anxiety, stress, and depression scale (DASS-42) [14] to determine their mental status before the test session. The explanation of the DASS has been mentioned in some references [14]. The normal ranges of DASS scores are as follows: depression (0-9), anxiety (0-7), and stress (0-14). The research was approved by Baqiyatallah University of Medical Sciences with The ethical code IR.BMSU.REC.1398.057.

Baseline cortisol level

At first, a minimum of 5 mL of saliva sample was collected from the participants for salivary cortisol level measurement. All samples were immediately cooled and stored at -80°C until analysis. Salivary cortisol concentrations were determined using a commercial enzyme immunoassay assay (LIA) with a specific salivary cortisol kit (ZellBio Co. Germany) according to the manufacturer's instructions. The concentration of free saliva cortisol was reported in ng/mL. The normal range reported by the kit and the proposed normal range is 5-21.5 ng/mL. The level of cortisol effect on brain activity should be evaluated when the EEG is studying [15].

Stroop Color and Word Test

All participants were in a room with moderate light, 40 cm away from a 13-inch monitor screen. The test process was explained to them before the test. In this test, the yellow, red, blue, and green words were displayed randomly at a speed of 60 frames per minute so that each word remained on the monitor for about 1 second. The words were in Adobe Erbic font and 65 font size.

The SCWT was a reliable test with variations in the color and number of the test items. However, the basic paradigm of the Stroop test has remained the same [16]. Firstly, each participant responded to 47 samples to get acquainted with the location of the blue, green, yellow, and red keys marked with a color bar on the keyboard. The test was then performed in 4 steps through 110 random repetitions showing the yellow, red, green, and blue words. In the first stage, the words were represented in white, and the participant must press a color on the keyboard based on the words' meaning. In the second stage, the words were shown in different colors so that the color of the word was according to the word's meaning. In the third stage, the words were not compatible with their color, and the participant had to choose the correct key based on the word's meaning. Finally, in the fourth stage, the words were not compatible with their color, but this time the correct response was the color of the words regardless of their meaning. To evaluate the effect of learning and repetition, the test was repeated 10 minutes later. The number of correct and incorrect responses and the correct responses' time delay were calculated at each step. The SCWT was designed and built in python software.

Electroencephalography recording

A 16-channel portable and wireless EEG device was used (Liv intelligent technology instrument, made in Iran) with the input impedance of 10 M, bandwidth: 2 kHz, gain: 1, and resolution: 24 bit. An EEG recording cap with silver/silver-chloride electrodes and LABVIEW software were used too. The amplifier was fixed behind the head. The electrodes' resistance was reduced to less than $15\text{ k}\Omega$ through cleaning the skin with alcohol and injecting a gel between the electrode and the skin. The electrodes were placed on the cap according to the international 10-20 system for EEG electrode placement. The ground electrode was placed on the middle of the head, and then the Cz was selected as the reference electrode. The data of 14 channels were recorded with a sampling frequency of 256 samples per second. The electrodes were placed at Fp1, Fp2, F3, F4, F7, F8, C3, C4, T5, T6, P3, P4, O1, and O2. First, EEG was recorded in the eyes-open resting state for 1 minute. Then, EEG was recorded for 1 minute during each stage of the SCWT. Linear and nonlinear features were extracted from EEG data by MATLAB software 2014. Pre-processing included the signal filtering between 1 and 50 Hz frequency bands, the visual inspection of each signal, and the removal of artifacts using the principal component analysis technique. The linear analysis was done by extracting the relative power of the standard frequency range. Relative band power is computed as the power of each frequency band over the sum of frequency bands. These frequency bands include (theta: 4-8 Hz, alpha 1: 8-10 Hz, alpha 2: 10-12 Hz, beta 1: 12-16 Hz, beta 2: 16-20 Hz, beta 3: 20-30 Hz, gamma 1: 30-40 Hz, and gamma 2: 40-50 Hz) [17]. The nonlinear indices included approximate entropy (AppEnt) (time-domain) [18], spectral entropy (SpEn) (frequency domain), Katz and Petrosian fractal dimension, and Hurst exponent and detrended fluctuation analysis [19].

Classification Method

In this study, an artificial neural network algorithm was used to classify four stages of the SCWT based on brain signals to determine the effect of the complexity of the pattern. The artificial neural network algorithm is one of the most critical and essential classification methods. Inspired by neural networks in the brain, this method creates a new structure for soft data processing. This structure comprises many unique processing elements called neurons that transmit data through synapses and coordinate to solve problems. The most critical point in these networks is their flexibility. If a small amount of incorrect or outdated data enters the system, that will

consistently deal with them. Learning in this system is adaptive, and synaptic weighting between neurons is done using repetitive methods. The main goal is for the system to predict the best response for new inputs.

Here, a two-layer neural network was used to classify the effect of the four-stage SCWT on brain signals. The network consisted of an input layer with 24 neurons and a hidden layer with 48 neurons. The inputs were linear and nonlinear features extracted from brain signals mentioned in the EEG recording section. The model's performance, reported in the result section, was evaluated by the iterative cross-validation method. In this part of the research, the artificial neural networks were implemented using Mat. Lab 2017b software.

Statistical Analysis

First, the 1-sample Kolmogorov-Smirnov test was done to test the normal distribution of variables in the data groups (stages and repetitions). If a variable had a normal distribution, the parametric tests were used, and if not, the non-parametric tests were used. To compare the number of correct and incorrect responses in the first and second repetition of the SCWT, the Mann-Whitney analysis was used to compare the stages. The Wilcoxon test was used to compare each stage in the first and second repetition. The two-way mixed model ANOVA was used to compare the time delay of correct responses, as a continuous quantitative index, between four stages and two repeats. Two-way mixed model ANOVA was used to compare linear and nonlinear EEG indices between two repetitions and four stages in each channel with a normal distribution. The Bonferroni test was used for pairwise comparison. Since the channels' changes are correlated together, we calculated the modified P value in the following website: <https://www.sdmproject.com/utilities/?show=FDR>, to ensure the accuracy and precision of the P value based on the false discovery rate method. The obtained significant level, less than 0.05, was also acceptable for the significant level.

3. Results

A total of 21 women and 19 men participated in this study. The Mean±SD age of men and women were 34±9 and 44±14 years, respectively. Characteristics of the participants (age, weight, DASS score, and pretest cortisol level) are listed in Table 1. The participants' cortisol levels were within the normal range based on the standard level of salivary cortisol reported by the ELISA kit. DASS scores of anxiety, depression, and stress were also

in the normal range. Therefore, not being in a stressful condition as one of the inclusion criteria was met.

SCWT Results

Table 2 presents the number of correct responses and time delays in the first and second tests. The Mann-Whitney and Wilcoxon non-parametric tests showed that the number of correct responses in the third stage with a significance level ($P < 0.05$) and in the fourth stage with a significance level ($P < 0.001$) was less than the first and second stages. In contrast, there was a significant difference between the first and second tests (Figure 1). The two-way mixed model ANOVA as a parametric test showed that the time delay of the correct response in the third and fourth stages of SCWT was significantly lower ($P < 0.001$) than the first and second stages. Moreover, there was no significant difference between the first and second tests (Figure 2).

EEG recordings results

There was no significant difference between linear and nonlinear indices in the test's first and second repetitions. There was a significant difference between resting state and SCWT in some linear indices, such as the relative power of alpha 1 (Figure 3) and gamma 2 (Figure 4). The relative power of alpha 1 during the test was reduced in all channels, compared to the resting state. On the other hand, the relative power of gamma 2 increased in all channels, compared to the resting state. The number of nonlinear indices, spectral entropy, Katz, and Petrosian fractal dimension failed to reach a significant level. However, they increased during the test compared to the resting state, especially in the O1 channel.

Classification

The model's evaluation results after cross-validation for each stage of the test are summarized in Table 3 for training, validation, and testing. The classification results are not considered here, indicating that these linear and nonlinear properties of the signals measured here by the mentioned method are insufficient to distinguish the effect of the four stages of the SCWT. According to Table 3, the amounts of accuracy obtained in the training, validation, and testing sets are not separate, which indicates that the model can be generalized to new samples and does not adhere to the trained data. Also, this multi-class model can better distinguish the second and fourth stages of the SCWT.

The Receiver Operating Characteristic (ROC) curves of the training, validation, and testing sets for each stage are demonstrated in Figure 5. The result shows that the

Table 1. Demographic Information of the Participants (N=40)

Variables	Mean±SD
Age (y)	39.2±12
Height (cm)	167.3±8
Weight (kg)	68.9±12
Pretest cortisol level (ng/mL)	9.8±6
Stress score	5.5±3
Anxiety score	3.9±3
Depression score	6.29±7

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test levels are not far from the midline, so the EEG indices' ability has not been enough in classifying the test stages. However, the testing set has more power to distinguish the fourth stage than the other stages.

4. Discussion

The present study aimed to evaluate brain activity based on linear and nonlinear indices of surface Electroencephalography (EEG) in the SCWT and investigate the effect of test repetition, with a short interval, on the responses and brain activity. The overall results of this study showed that the activity of the brain during the SCWT changes compared to the resting state so that the relative power of the alpha frequency band decreases in the frequency range of 8-10 Hz and the relative power of the gamma frequency band increases in the frequency range of 40-50 Hz. It is worth mentioning that the results of one study in 2017 provide good support for the present study results, according to which the alpha band

decreased and the gamma band increased during the test, compared to the resting state [10]. Several studies showed that EEG recording during the SCWT significantly differs from the resting state. Increased gamma and beta bands are indicators of enhancing top-down and bottom-up attention [20]. A high gamma band can more effectively reflect the emotional difference than a low gamma band [21]. The results did not show a significant change in beta activity similar to nonlinear features. It has been found that changes of the nonlinear indices were according to the changes of the relative power of the beta band frequency [22]. Thus the SCWT is a mental effort test than a cognitive test [23].

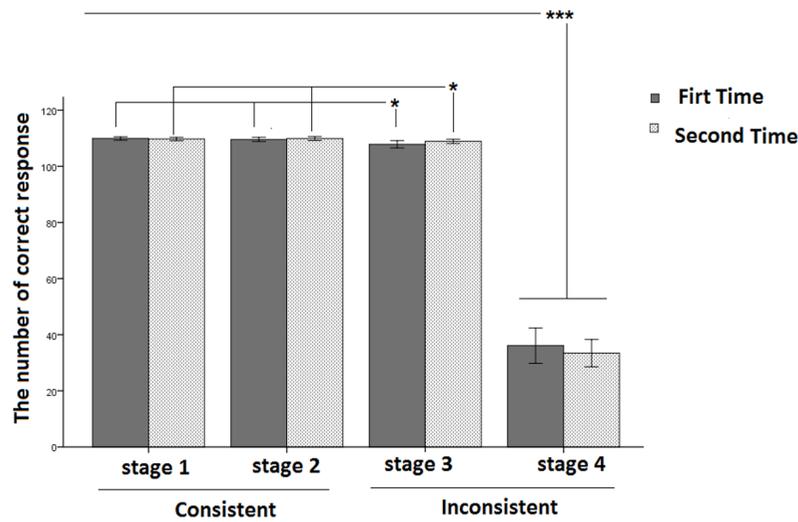
According to our results, although there was a clear and significant difference in terms of the correct responses and their delays between the stages of 3 and 4 and the stages of 1 and 2, there was no significant difference between various test stages, from easy to challenging stage. There were no indices to distinguish between various

Table 2. Mean±SD of the number of correct responses and their delay time in four stages of stroop test in the first and second time

Stages of SCWT	Mean±SD			
	First Repetition		Second Repetition	
	The Number of Correct Responses	Responses' Time Delay	The Number of Correct Responses	Responses' Time Delay
Stage 1	109.7±1.9	0.885±0.17	109.7±1.7	0.792±0.11
Stage 2	109.4±2.2	0.849±0.15	109.9±2	0.764±0.14
Stage 3	107.9±3.5#	1.032±0.25**	108.8±2#	0.9790.27±**
Stage 4	35.6±17*	1.045±0.8**	33.4±14*	0.976±0.23**

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* P<0.0000001 between stage 4 and other stages. # P<0.05 between stage 3 and stage 1 and 2. ** P<0.001 between stages 3 and 4 and stages 1 and 2.



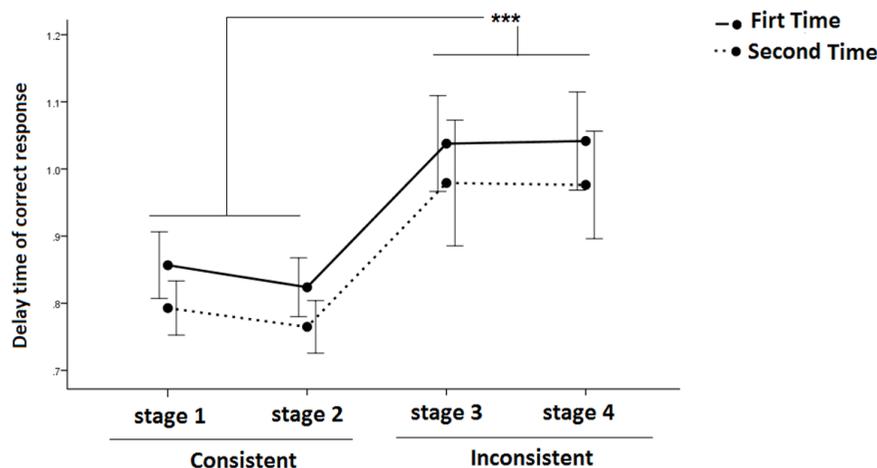
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Figure 1. The significant differences in the number of correct answers between the third level of the SCWT and the first and second levels, and between the fourth level of the SCWT and the previous three levels

*** $P < 0.0000001$; * $P < 0.05$.

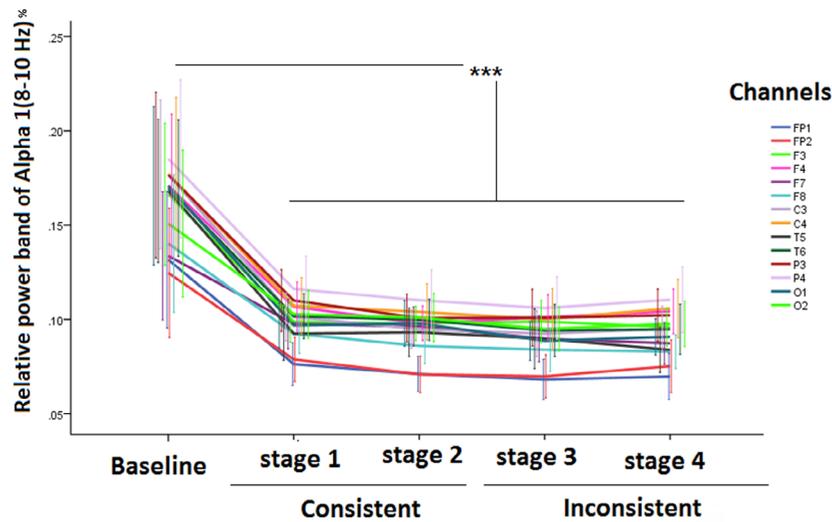
stages of the SCWT. The decrease in the correct responses in the fourth stage results from the method. Participants had been trained to find the correct answer based on the meaning of the word in stages 1 to 3, and suddenly, they were asked to respond to the color of a word in the last stage. Another point to mention is that while we recorded each stage of the test for 1 minute, other studies reporting differences between various stages of the SCWT did the very analysis by comparing evoked response potential (ERP). The brain activity changes after stress. Several studies showed a marked difference in the linear and nonlinear indices, brain connectivity, and networks of EEG recording, especially in the alpha activity

and Katz fractal dimension between pre-stress and post-stress conditions [24-26]. However, they did not examine the difference between the SCWT stages, although the SCWT is considered a stress inducer test [13]. According to these studies, there is a strong positive correlation between all EEG sub-bands and the consistent SCWT in the forehead. Besides, in inconsistent SCWT, there is significant positive connectivity between the frontal and temporo-frontal regions [11]. Another study showed that the inconsistent test increased the negative peak intensity in the frontal and central regions. In other frontal and parieto-occipital areas, the positive peak was amplified [27]. Studies have also shown that the theta and alpha



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Figure 2. Significant difference between delay correct response between the inconsistent stages and the consistent stages of SCWT ($P < 0.001$)



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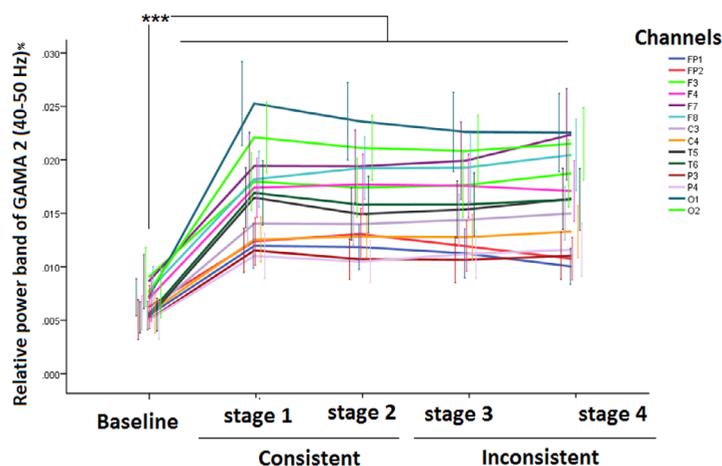
Figure 3. Significant decrease in the relative power of alpha 1 band frequency (7-10 Hz) in SCWT compared to the rest time ($P < 0.00001$) in all EEG channels

frequency bands' activity significantly decreased in cases with incorrect responses [7]. They concluded that an increase in error is associated with mind-wandering accompanied by a decrease in theta and alpha activity [7].

Regarding brain connectivity assessment, various studies indicated that the frequency band between 13 and 20 Hz is sensitive to the consistent/inconsistent SCWT. In terms of mismatch, a high correlation was observed between the left frontal and left parietal regions. The faster the individual responded, the more significant the right frontal-parietal region's correlation in the matched response [12]. Brain imaging studies have shown that the Anterior Cingulate Cortex (ACC) is markedly activated in mismatched conditions. At the same time, this area's

activity is associated with the activity of the prefrontal cortex (PFC) to prepare for the next test [27]. In another study, it has been observed that the lower PFC and the left parietal cortex are involved in the information processing of this type of SCWT. In contrast, the ACC cortex does not show any specific activity [28]. In another study, the frontal lobe and anterior cingulate cortex activity significantly increased in the SCWT [29].

Therefore, the study results do not confirm the above results due to the difference in the method, stating no significant difference in the electrical activity of the brain in terms of relative band power and signal complexity in both consistent/inconsistent tests. None of these indices can distinguish the four stages of the SCWT. However,



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Figure 4. Significant increase in the relative power of the gamma 2 band frequency (40-50 Hz) in the SCWT compare to the rest time ($P < 0.00001$) in all EEG channels

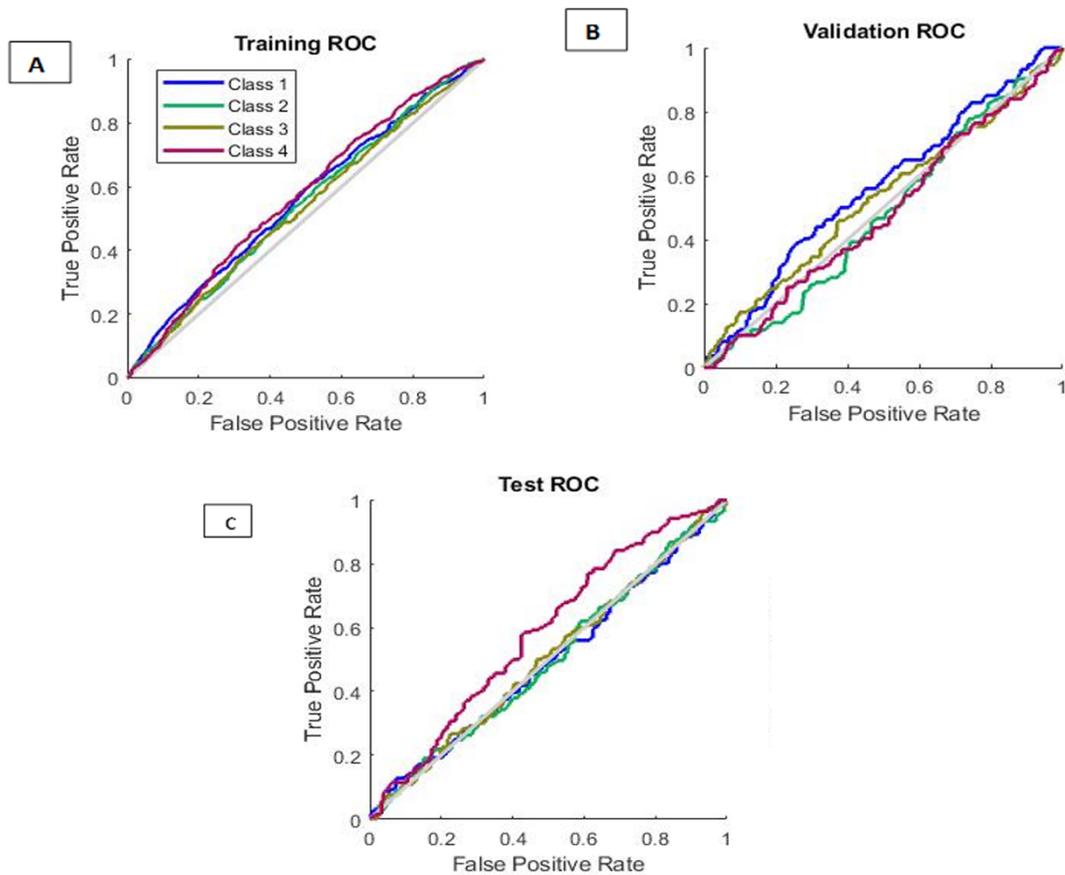


Figure 5. Curves for each training set (A), Validation (B), and Test (C) of EEG indices for classifying SCWT stages

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there was a significant difference between matched and mismatched stages in terms of the response delay and the number of correct responses. In a similar study by West et al., a 1-minute EEG recording of each consistent/inconsistent stage of the SCWT was performed. This study's results also could not show any significant difference in the relative power of frequency bands between various stages of the test [10]. The main limitation of this study was the method of recording brain signals, which

was not ERP. Therefore, it is suggested to use the ERP registration method in future studies.

5. Conclusion

This study showed that the number of correct responses in the third and especially fourth stages was less than the first two stages of SCWT, and the delay of correct responses was more. One-minute EEG activity was not significantly different between various stages of SCWT.

Table 3. Results of artificial neural network model for classifying stroop test levels based on EEG features

Data	Mean of Accuracy (%)				
	Stage 1	Stage 2	Stage 3	Stage 4	Total
Training	27.6	41.7	15.1	29.2	28.7
Validation	29.9	35.6	17.3	18.5	25.3
Test	20.6	36.5	15	32.1	26.4
All	26.8	40	15.4	28.2	27.8

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There was no significant difference between the first and second test repetitions in terms of the responses, time delay of the responses, and brain activity in all bands at various stages. Another finding of this study revealed that the alpha 1 band's relative power significantly decreased during the SCWT compared to the resting state. The relative power of the gamma 2 band increased. At the same time, no significant difference was observed in other linear and nonlinear indices.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the University Human Research Ethics Committee, and all procedures involving human participants were performed following the ethical standards. The research was approved by Baqiyatalah University of Medical Sciences with ethical code IR.BMSU.REC.1398.057.

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Authors' contributions

All authors passed the four criteria for authorship contribution based on the international committee of medical journal editors (ICMJE) recommendations.

Conflict of interest

The authors declared no conflict of interest.

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