Video Article

Using Brain Activation (nir-HEG/Q-EEG) and Execution Measures (CPTs) in a ADHD Assessment Protocol

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Abstract

Attention Deficit Hyperactivity Disorder (ADHD) is a problem that impacts academic performance and has serious consequences that result in difficulties in scholastic, social and familial contexts. One of the most common problems in the identification of this disorder relates to the apparent over diagnosis of the disorder due to the absence of global protocols for assessment. The research group of School Learning, Difficulties and Academic Performance (ADIR) from the University of Oviedo, has developed a complete protocol that suggests the existence of certain patterns of cortical activation and executive control for identifying ADHD more objectively. This protocol takes into consideration some of the hypothetical determinants of ADHD, including the relationship between activation of selected areas of the brain, and differences in performance on various aspects of executive functioning such as omissions, commissions or response times, using innovative tools of Continuous Performance Testing (based on Virtual Reality CPT and Traditional CPT) and brain activation measures (two different tools, based on Hemoencephalography- nirHEG; and Quantified Electroencephalography --Q-EEG, respectively). This model of assessment aims to provide an effective assessment of ADHD symptomatology in order to design an accurate intervention and make appropriate recommendations for parents and teachers.

Video Link

The video component of this article can be found at https://www.jove.com/video/56796/

Introduction

The overall goal of the present protocol is to develop a complete procedure or model of assessment for the diagnosis of Attention Deficit Hyperactivity Disorder, otherwise known as ADHD. ADHD is one of the problems that impacts academic performance. It is understood to be a disorder characterized by problems with attention, inhibitory control and hyperactivity, whose performance is significantly lower than their peers[1,2]. The latest version of the Diagnostic and Statistical Manual of Mental Disorders (DSM) [1] includes different updates from the previous version: ADHD has been categorized as a neurodevelopmental disorder; the age of appearance of the symptoms has been increased so now the symptoms can manifest before 12 years of age; the term ADHD presentation should be used instead of subtype (predominantly hyperactive/impulsive; predominantly inattentive; and combined presentation, and); finally, it has been accepted as a comorbidity with autism spectrum disorders.

There are different estimations of prevalence rates depending on the country or region analyzed[3,4,5]. An international global systematic[3] review observed an average prevalence rate of 5.29%. However, applying criteria from the Diagnostic and Statistical Manual of Mental Disorders [4], the percentage ranges from 5.9 to 7.1%. Similarly, a meta-analysis of ADHD in a Spanish population provided an average of 6.8%[5]. The variations in prevalence rates could be due to the different assessment protocols used.

Although there is a considerable body of research suggesting a neurological basis for ADHD, the origins of this disorder remain unclear. Several studies have associated the ADHD symptomatology to brain cortical hypoactivation, which is related to a deficit in the dopaminergic and noradrenergic systems[6]. The noradrenergic system modules the selective attention and the activation levels needed to carry out a task. On the other hand, the dopaminergic system is responsible for inhibitory control, both at an executive and motivational level. The low cortical activation related to the dopaminergic and noradrenergic systems is presumed to be the basis for the inhibitory and attentional deficits presented in the ADHD. In this sense, children with ADHD show low cortical activation in the dopaminergic and noradrenergic systems, which is manifested by different profiles of electro cortical activity in a state of rest, evidenced by increased theta -and decreased beta- activity[7,8] as well as low levels of blood oxygenation in the Fp1 (front left side of the frontal lobe), and FPz/Cz (central zone of the pre-frontal cortex) regions.

Blood oxygenation is measured using nir-HEG, which uses functional near-infrared spectroscopy to measure color changes in the blood in the brain to indicate oxygen saturation areas; oxygenated blood is bright red whereas de-oxygenated blood is a deep, almost purplish crimson. Cortical activation is measured using quantified electroencephalography (Q-EEG). This is a computerized EEG system that records electrical activity in the brain to provide levels of cortical activation through the beta/theta ratio. It measures attention in general, independently of the task being performed. Other studies have focused on the existence of an executive function (EF) impairment in the ADHD population[9], which would explain the difficulty children with ADHD have controlling impulsive responses, resisting interference or distraction, organizing activities in a sequential manner, and sustaining cognitive effort while performing an activity.

Generally, these characteristic symptoms of ADHD have serious consequences which result in difficulties in scholastic, social and familial contexts. Children with ADHD have a higher probability of repeating a grade and/or completing fewer grades at school than children without ADHD. Moreover, dropping out of high school is three times more likely among youth with ADHD[10,11].

Considering the modifications and the new categorization of ADHD in the current version of the Diagnostic and Statistical Manual of Mental Disorders [1], results relevant to establish the relationship between cortical activation levels in specific brain areas, executive functions, and diagnosis-related variables[7,8,11,12] (*i.e.*, differences between the three types of ADHD presentations).

One of the most common problems in the identification of ADHD is the over diagnosis of the disorder due to the absence of global protocols for assessment. The fact that professionals do not have a general protocol based on objective variables is causing a large percentage of false positive and false negative cases of ADHD. This situation highlights the need for professionals and clinicians to have a clear protocol that considers not only the relevant variables but also the relationships between them.

For this reason, the research group of School Learning, Difficulties and Academic Performance (ADIR) from the University of Oviedo has been working on developing a complete protocol to identifyprofiles of cortical activation and executive control to provide a more objective diagnosis of ADHD than what is currently in use. This protocol is particularly important because it takes into account the fact that cortical activation in the frontal and prefrontal cortex impacts the executive function. The current protocol will be useful for clinicians who are interested in performing a complete assessment that considers the interaction between relevant variables in the diagnosis. To that end, the protocol is based on the assessment model from a recent study proposed by Rodriguez *et al*. which takes into consideration the interaction between cortical activation and executive variables (**Figure 1**).

In summary, the purpose of this protocol is to provide a more objective diagnostic procedure for this developmental disorder than is currently available, and to analyze in depth the relationship between activation measures and executive function measures. The procedure will also take into consideration some of the hypothetical determinants of ADHD, both in the relationship between activation of selected areas of the brain and differences in performance on various aspects of executive functioning such as omissions, commissions or response times.

Protocol

The present study was conducted according to the Declaration of Helsinki, which establishes the ethical principles for research involving human beings. The study's aims, scope and procedure were also approved by the Ethics Committee of the University of Oviedo and University Hospital of Asturias.

1. Parents Report

1. Before starting the assessment protocol, obtain signed parental consent for the child to be assessed.
2. Conduct a structured interview with families of the participants.
   NOTE: The structured interview has been provided as an appendix.
3. Have families and/or teachers of the participant complete the Scale for the Assessment of ADHD[13]. This scale comprises 20 items that provide information on the presence of symptoms related to attention deficit and hyperactivity/impulsivity that are referred in the Diagnostic and Statistical Manual of Mental Disorders.
   1. In this section, have the therapist give the following instructions: "Now, in order to get significant information about your child, you must complete this questionnaire referring to the symptoms that might be representative of your child's behavior. You must classify the frequency with which you observe these 20 symptoms".

2. Cognitive Measures

1. To measure the attentional variables, administer a cognitive scale.
   1. Administer a Cognitive Scale[14] (following the protocol in the manual) for assessing individual intelligence in children and adolescents between the ages of 6 years and 16 years 11 months.
   2. Analyze in depth the cognitive profile obtained by the scale.
      NOTE: Considering the information included in the cognitive scales manual, the results from the Wechsler scale could be influenced by ADHD, leading to lower scores in processing speed and working memory in comparison with perceptual reasoning and verbal comprehension.

3. Executive Measures Using Continuous Performance Tests

1. Analyze the performance of the children using two CPTs: one based on a Virtual Reality CPT[15] and the other consisting of a Traditional CPT[16].
2. Administer the virtual-reality based CPT[15]. This is a CPT based on a virtual reality environment that reproduces the conditions of a regular classroom. It evaluates attention, impulsivity, processing speed, and motor activity in children and adolescents between 6 and 16 years of age. The activities that make up the test are preset, and it is not possible to change any specific characteristic of the tasks.
   1. Double-click on the Virtual Reality CPT app to start the program.

2. Enter the username and password provided by the program license.
3. Enter the participant information (name, surname and date of birth) and the click on the "Go into the virtual classroom" window.
4. Ask the participant to wear the Head Mounted Display (HMD) glasses and headphones, and inform them of the importance of wearing it during the whole test. Have the therapist say: "Now you need to wear these special glasses during the virtual reality tasks, if you don't see very well or have any issue about that, please tell me and I help you to resolve it".
   NOTE: These glasses are connected to the PC, so the therapist can see the images the participants are looking at in order to ensure that they understand the test (**Figure 2**).
5. Ask the participant to hold the button with their dominant hand before starting the virtual attentional tasks. Have the therapist say: "You need to hold and press the button with the same hand you write with".
6. Have the participant explore the virtual environment (a typical classroom) and take the perspective of a student sitting at one of the desks looking at the blackboard for 15 s (**Figure 3**). Conduct this part by the virtual teacher though these instructions: "Hello, with the glasses that you are wearing, you can see the entire classroom: to the left, to your right, up, and down. You can see everything. Notice all the things in the room, look at the walls and the other people, look at whatever you want".
7. Have the participant follow the virtual teacher, who guides them through the tasks. The first part the participant performs is training, which consists of visually locating balloons and popping them by pressing the button. Have the virtual teacher in this part instruct this part as follows: "Now that you have inspected everything, you're going to start doing something fun. We have placed some shiny red balloons around you. You need to find the balloons by moving your head. When you find one, look at it closely, and press the button to make it pop. You have to find 4 balloons. Let's go! The challenge starts now!"
8. Have the participant continue to the next step which is the first exercise. This is based on the "x-no" paradigm (traditionally known as "no-go"), where the participant must press the button when he or she does not see or hear the stimulus "apple". Have the virtual teacher say: "Now, you will to see some drawings on the chalkboard. I will also say a few words to you. You have to pay attention and click the button, AS FAST AS POSSIBLE, when you see or hear anything THAT IS NOT AN APPLE. But be careful! You need to press the button every time you see or hear any other thing. For example, if you hear "CLOUD" or if a drawing of a cloud appears on the chalkboard, you have to press the button. Remember: Never press the button when you see or hear apple! The challenge starts... Now!".
   Note: As the activities are preset by the program, it is not possible to change the target-stimulus (in this case, APPLE).
9. Have the participant complete the last exercise by following the instructions to press the button whenever he/she sees or hears the number "seven". This part consists of a "X" paradigm (or "go" task). Say these instructions: "Now you are going to work with numbers. You will see numbers on the chalkboard and you will also hear numbers. You must press the button every time you hear or see the number "SEVEN". DO NOT press the button for any other number. For example, if you hear or see the number "THREE" DO NOT press the button. Remember: Always press the button when you see or hear "SEVEN"! The test starts now!"
   NOTE: As the activities are preset by the program, is not possible to change the target-stimulus (in this case, SEVEN).
10. Print the report of the test results via the test website adding the name and surname of the child assessed. This report compiles results for the following variables: omissions, commissions, response time, and variability and complement this information by differentiating these measures of sensory modality (visual vs. auditory), presence/ absence of distractors, and task type (go vs. no-go), thereby leading to different execution profiles.

3. Administer the Traditional CPT[16]. This is a CPT that can be used in a visual or auditory version, but in this protocol only the visual version is used. This test contains preset tasks. The visual norms for CPT go from 4 years to more than 80, by age and gender.
   1. Make sure that the participant is relaxed, paying attention to their breathing and ensuring that it is controlled. If it appears that the participant is not controlling their breathing. Indicate as follows: "You should focus on abdominal contraction and controlled breathing out".
   2. Open the CPT application and enter the name and birth date of the child, and press the okay button.
   3. Click on visual tasks window and click the **launch** button, the computer will start to load the visual tasks.
   4. Ensure the participant is holding the button (which is connected to a PC) with his/her dominant hand. Repeat the instruction in step 3.2.5: "You need to hold and press the button with the same hand you write with".
   5. Inform the participant of the following instructions for the test: "You have to press the button when you see a black square in the upper part which is the target stimulus. However, do not press the button when the black square is in the bottom part" (**Figure 4**).
   6. Have the participant perform the training part (which lasts for approximately 3 min) until it is clear the participant understands the task. In the training part of the CPT, the target (stimulus to which the participant has to respond) and the non-target (stimulus to which the participant has to avoid responding) stimulus appear randomly in the screen; the therapist should observe whether the participant presses the button correctly or not. If the participant does not press the button according to the instructions, have the therapist explain the instructions again and then the participant will need to repeat the training section.
   7. Tell the participant that the task is very long and that she/he must keep still and concentrate on doing the tasks.
   8. Have the participant complete the CPT activities. It is divided into two sections. The first section of the test (the "Infrequent" or vigilance mode), is characterized by target: non-target ratio of 1:3.5. This is evidenced by a tendency to not press the button. In the second section (the "Frequent" or high response demand mode) presents a target:non-target ratio of 3.5:1, so the child must inhibit the tendency to respond.
   9. After completion of the CPT, generate the report which compiles the following variables: omissions, RT (response time between the presentation of target stimulus and subject response), commissions, variability, D prime (quality of concentration during the test based on the number of errors in the test) and ADHD Index, attributable to a presence or absence of attention deficit with hyperactivity. Calculate this ADHD Index from the sum of: TR of first half + D prime second half + total variability and is interpreted as attributable to ADHD when the score is lower than -1.80.

4. Cortical Activation Measures in Fp1 and Fpz/Cz Regions (Hemoencephalography, and Quantified Electroencephalography)[17]

1. Open the Bioexplorer software and choose the design **POCKET_HEG_video_1ch_AI_HEGRatio.bxd**.

1. Press **time on** button to see the time during the assessment (35 s).
2. Inform the participant that he/she cannot move and must be still. The therapist informs the participant about the importance of remaining still during the assessment of blood oxygenation: "Now it is very important that you are still during the assessment of blood oxygenation because if you move, the system will fail, and it will be necessary to measure again. Are you ready?"
3. Click on the **red button** and enter the name of the subject and the cortical point which is going to be measured (Fp1 region for assessment of inhibition capacity and Fpz for the assessment of attention capacity).
4. Put the band on the participant's forehead in a specific area: Fp1 (for assessment of inhibition capacity) and Fpz (for the assessment of attention capacity) as can be seen in **Figure 5**.
5. Make sure that no external light enters the band. Properly adjust the band to the participant's head in such a way that it is not loose and does not fall off during the evaluation.
6. Put the Hemoencephalography-nirHEG (which is connected to the headband) around the participant's neck, like a necklace.
7. Turn on the hardware to connect the band to the PC.
8. Measure the blood oxygenation of a specific area through the nir-HEG program for approximately 35 s. Press the **green button** to start to measure blood oxygenation and 35 s later press the **white button** to end the assessment.
9. Print the output provided by the program and analyze the nir-HEG Ratio of the participant provided by the program. The mean of this tool is 100 (SD = 20) which is used to calibrate the spectrophotometers. An Attention Index (AI) is also obtained through the nir-HEG , which shows the incapability of the participant to increase the ratio and brain activation. This is useful to identify attentional problems and measure capacity of concentration.

2. Measure the cortical activity levels of the participant using the quantified electroencephalography (Q-EEG), a computerized EEG system that provides levels of cortical activation through the beta/theta ratio. It is used to measure general levels of attention, not taking the performed task.
NOTE: The analysis of beta/theta ratio have been carried out according to previous studies[12].
    1. Inform the participant of the correct abdominal breathing required during the test.
    2. Open the Bioexplorer software and choose the design **combined_Theta_Beta.bxd**. The screen will appear divided into two windows (the left side shows the cortical activity of FP1 and the right side shows the cortical activity of the CZ)
    3. Click on the **red button** and enter the name of the subject and the cortical point which is going to be measured (Fp1 region for assessment of inhibition capacity and Cz region for the assessment of attention capacity).
    4. Put the red electrode in the Fp1 region and the blue electrode in the Cz region, applying electrode gel (using the information found at: "http://neurologiclabs.com/neuromonitoring/eeg/", to ensure that the electrodes are correctly placed) (see **Figure 6**).
    5. Put two more control electrodes (the black and white electrodes) on the participant´s left and right earlobes, applying electrode gel.
    6. Ensure the participant has her or his eyes open during the assessment. The therapist must watch the participant throughout the assessment. If the participant closes their eyes at any time, end the assessment process and repeat it once the participant is rested.
    7. Place an electromyography (EMG) sensor on the right forearm to identify the degree of movement.
    8. Inform the participant that he/she must remain relaxed, trying not to move during the task an breathing slowly. The participant must be concentrated on the computer screen while the theta and beta waves are being emitted. The therapist says: "You must be still and relaxed for 35 s, the only thing you need to do is to watch the theta/beta waves that will be appearing on the computer screen".
    9. Press the **green button** to start to measure cortical activity, and 35 s later press the white button to end the assessment.
    10. Print the results generated from program and analyze them. If the beta/theta ratio is lower than 50% at Cz, this evidences a deficit of sustained attention. On the other hand, if the ratio is also lower at Fp1, this result is related to an impairment of executive control, attributable to hyperactivity.

Representative Results

Using the assessment procedure presented here, it is possible to carry out an effective assessment about ADHD symptomatology in order to design an accurate intervention and make recommendations for parents and teachers. Below are a series of representative results of a participant with ADHD and a participant without ADHD, which will allow professionals to see the differences between the two profiles.

Once clinicians had the informed consent from the families, a cognitive scale (WISC IV) was administered to the children in order to exclude those participants who present low or high capacities. The following steps then compiled an attentional profile of the children using the Continuous Performance Test and Activation Cortical Techniques (Q-EEG and nir-HEG). **Figure 7** shows the results of children with and without ADHD in the Virtual Reality CPT. These results show the children with ADHD have more omissions and commissions errors, as well as higher motor activity and larger response times.

Similarly, **Figure 8** shows the results obtained by the Traditional CPT showing how the child with ADHD presented larger percentages in omissions, commissions, response times and in the variability of the response. While the child without ADHD showed the best scores at the end of the tasks, the child with ADHD did not show improvement in any of the four blocks.

Figure 9 shows an example of the Cortical Activation collected by nir-HEG in a child affected by ADHD who obtained 24.5 percentage points below the average in Fp1 region.

Similarly, an example of the measures collected by Q-EEG in a child with ADHD (FP1 region) are shown in **Figure 10** that evidences how the symptoms of ADHD cause a decreased in cortical activation (ratio beta/theta under 0.5).

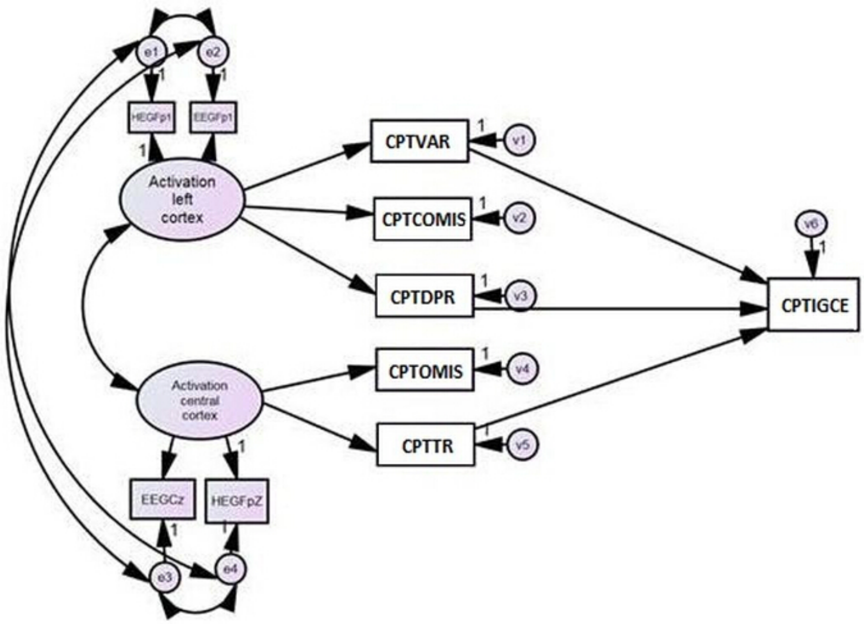

Figure 1. Model of ADHD assessment
Based on Hemoencephalograpy (nir-HEG), Quantified Electroencephalography (Q-EEG), and Traditional CPT (CPT). Variables included: HEG-Fp1(nir-HEG ratio from left pre-frontal cortex); HEG-FpZ (nir-HEG ratio from central pre-frontal cortex); Q-EEG-Fp1(beta-theta ratio from left pre-frontal cortex); Q-EEG-CZ (beta-theta ratio from central cortex); CPT-OMIS (omissions committed in CPT); CPT-COMIS (commissions committed in CPT); CPT-VAR (Response Variability during CPT tasks); CPT-RT (Response Time obtained in CPT); CPT-DPR (D prime Index provided by CPT); ADHD-INDEX (ADHD Index provided by CPT). This model reflects a stronger association between activation (central and left prefrontal) and execution in ADHD children than their peers without the disorder. Low cortical activation (by QEEF) and blood oxygenation (by nir-HEG) in Fp1 region, are related to low performance in CPT. Similarly, normal levels of electrical activation and blood oxygenation are associated to normal results from CPT. Provided by Rodríguez *et al.*[11]. Please click here to view a larger version of this figure.

Figure 2. Head Mounted Display(HMD) glasses of the Virtual Reality CPT.
The image shows the Head Mounted Display (HMD) glasses, the headphones and the button which must be held with the dominant hand. The image also shows the virtual classroom which is shown to the participant via the glasses and to the therapist via the computer screen. Please click here to view a larger version of this figure.

Figure 3. Virtual classroom provided by the Virtual Reality CPT.
Virtual classroom environment where the participant does the tasks which are explained by a virtual teacher. Please click here to view a larger version of this figure.

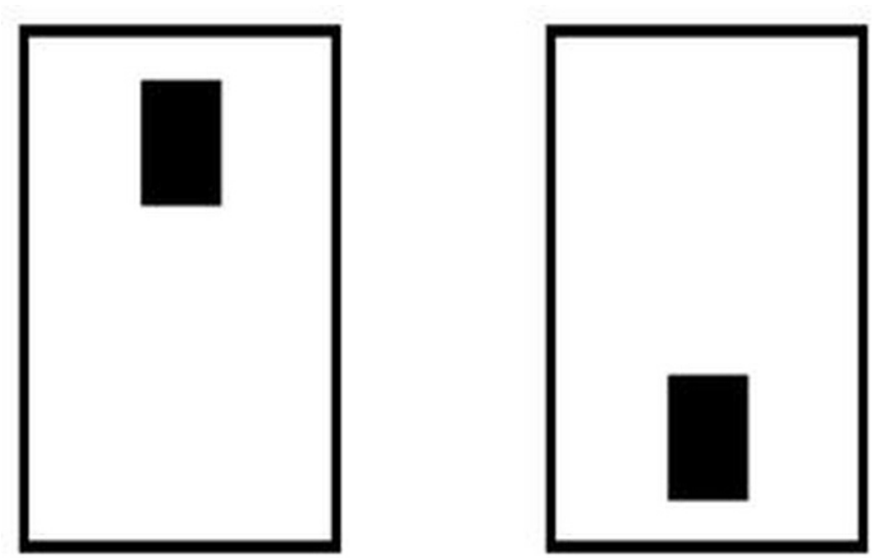

Figure 4. Images present in the Traditional CPT.
The right-hand picture shows the non-target stimulus and the left-hand picture shows the target stimulus. Please click here to view a larger version of this figure.

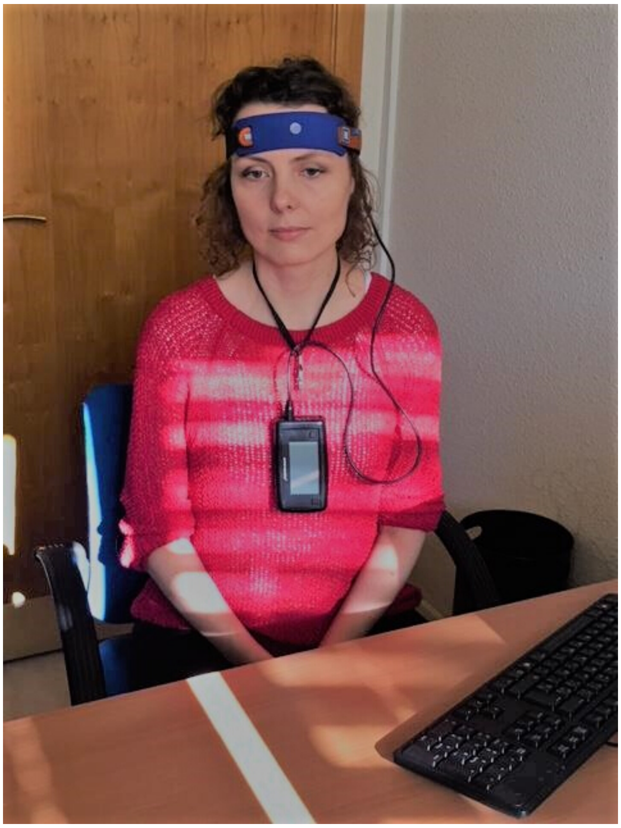

Figure 5. Participant wearing the nir-HEG band.
The image shows the nir-HEG band placed in the Fp1 region and the hardware connected.

Figure 6. Participant with electrodes of Q-EEG.
The image shows the blue electrode in the Cz region and the red one in the Fp1 region. The control electrodes: black and white, have been placed on the participant´s left and right earlobes, applying electrode gel. Finally, an electromyogram was placed on the right forearm.

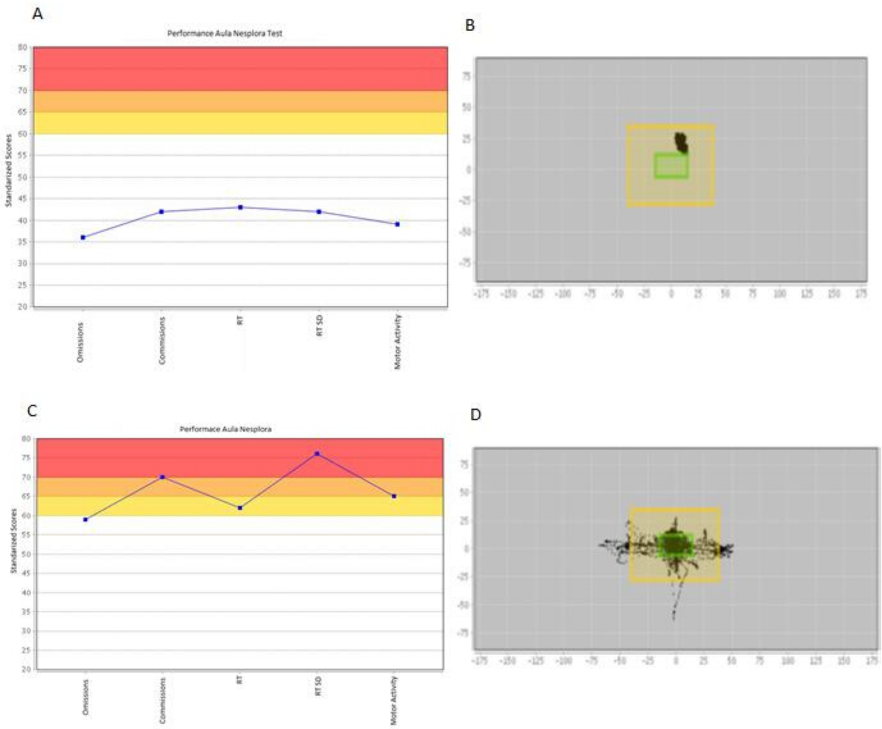

Figure 7. Performance and motor activity during the Virtual Reality CPT.
The Y axis shows the standardized scores from the Virtual Reality CPT (scores above 60 mean low performance in the variable studied and scores below 60 mean good performance of the variables studied. The X axis shows the different variables provided by the Virtual Reality test. The colors: yellow, orange and red, represent the grade of severity: low, medium and high in the performance of different variables. A and C illustrate the performance of a participant without ADHD (A) and a participant with ADHD (C).
Images B and D represent the subject´s head movement (motor activity) throughout the test. The green square refers to the blackboard and the yellow square represents the area in which the virtual blackboard can be inside the visual range for detecting the stimulus. If child move out of the yellow square, it makes impossible to perform the visual task properly. The dot diagram provides a graphic which represents the child's attention towards the blackboard and to the general task. Image B represents the motor activity in a participant without ADHD and image D represents the motor activity of a participant with ADHD.

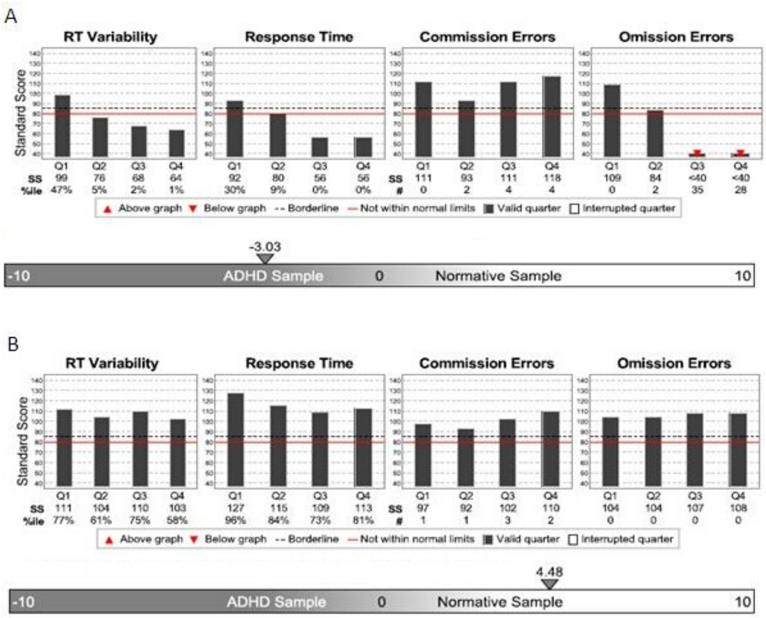

Figure 8. Profile provided by the Traditional CPT in the main variables.
Figure A represents the profile of a student with ADHD while Figure B shows the execution of a child with a performance similar to the normative group. The term "SS" refers to Standardized scores. Standardized scores below 80 represent low performance. "Q1"(25%), "Q2"(50%), "Q3"(75%) and "Q4"(100%) represent the four quartiles of the CPT tasks. This division is useful in order to see whether the subject's attention decreases during the activities or not.

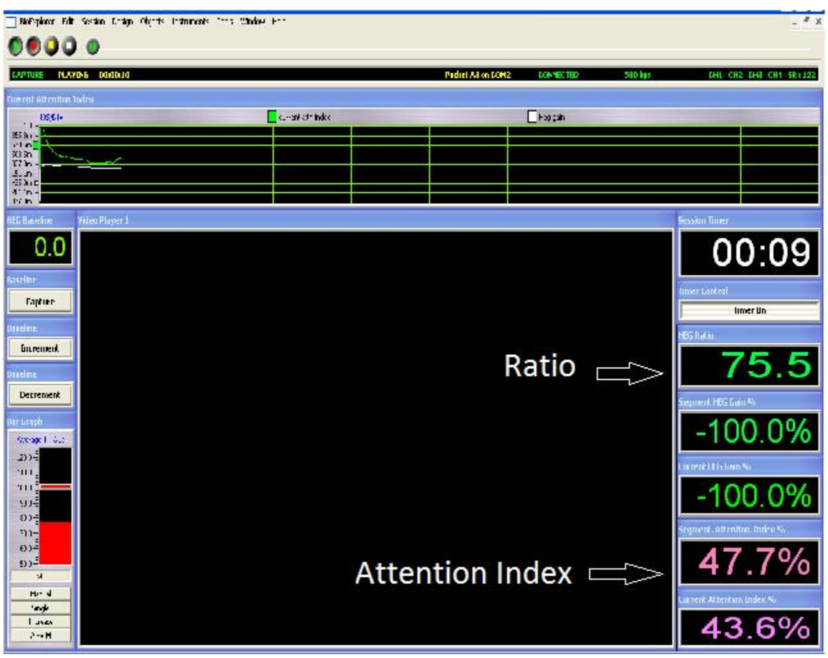

Figure 9. Activation measure with the nir-HEG.
This Figure shows the ratio indicator of the oxygenation of the blood at specific point (Fp1/Fpz) and an attention index which is expressed as percentage (a percentage below 50% represents a low attentional index).

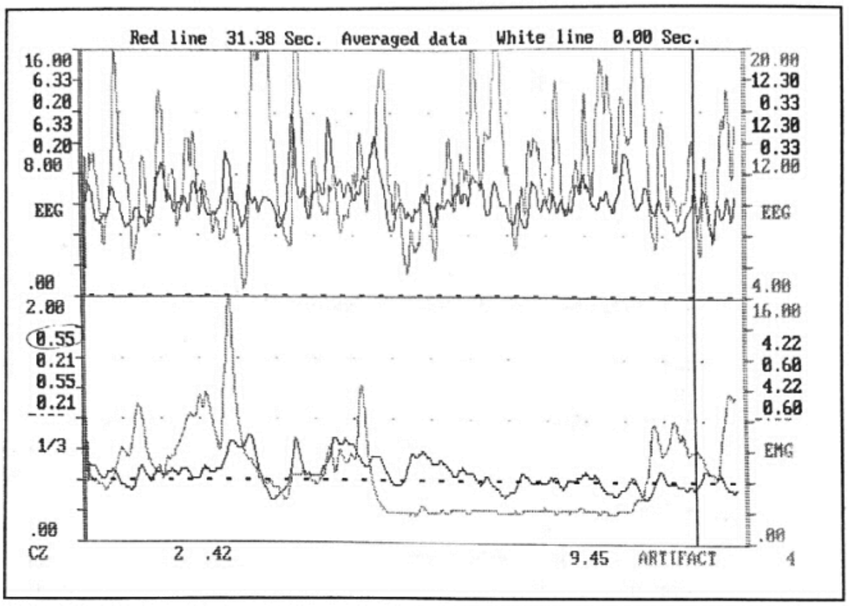

Figure 10. Cortical activation measures in FP1.
The Figures show the output from Q-EEG. The top part shows beta/theta waves measured individually, and the bottom part shows the beta/theta ratio in the Cz region and electromyography results from the right forearm. The CZ beta/theta ratio is shown on the left (in this case is 0.55), cortical activation is good when the ratio is above 0.50. The electromyography from the right forearm is used to ensure that the participant is relaxed at a muscular level, and is on the right (in this case the value of EMG is 4.22). Good values in electromyography are below 5.0. Please click here to view a larger version of this figure.

Discussion

Here we present an effective protocol for assessing ADHD from 6-16 years of ages. Given the symptomatic complexity of ADHD and its high prevalence rates, professionals must have reliable and valid instruments to diagnose this disorder. Generally, questionnaires based on behavioral observations are widely used. However, the use of these instruments as the sole assessment measure has certain limitations, including potential subjectivity on the part of the observer[18].

As the results show, this protocol placed emphasis on highlighting the differences between a participant with ADHD against a participant without ADHD. More specifically, it could be seen that a participant with ADHD had lower scores in attentional variables from both CPTs (omissions, commissions, response times and motor activity) as well as lower cortical activation and blood oxygenation in Fp1 and Fpz/Cz brain regions. For this reason, it is very relevant to contrast the information obtained by observation scales with the cognitive profile of the children and the performance in CPTs. Professionals can then perform a more realistic and reliable assessment and, thus provide recommendations for parents and teachers that are more specifically adapted to the individual needs of each child.

However, a critical step of this protocol is the management of exclusion criteria; professionals must ensure the ADHD symptoms are not due to another cause such as perceptive, emotional or social problems[19].

A minor limitation of this protocol is the time required to complete each assessment. Generally, this protocol is best divided into several sessions to ensure participant well-being. The applicability of this method has been presented in previous studies[11,12] which showed the effectiveness of the assessment model for getting an accurate diagnosis of ADHD according to the Diagnostic and Statistical Manual of Mental Disorders criteria.

Future lines of research would be based on the inclusion of neurological tools for getting an objective assessment of ADHD, considering functional and structural neuroimaging methods. Although, this research used quantitative analysis from Quantified Electroencephalography and Hemoencephalography (nir-HEG), it would be positive the inclusion of other instruments to contrast neuropsychological measures. Moreover, this type of study helps the clinicians to detect specific profiles of cortical activation (Q-EEG) and CPT (executive control) to differentiate the three types of ADHD presentations.

Disclosures

The authors who are listed above certify there are no financial interests or other conflicts of interest regarding the present study.

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