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Super-oxide anion production and antioxidant enzymatic activities associated with the executive functions in peripheral blood mononuclear cells of healthy adult samples

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ABSTRACT

Executive Functions (EFs) involve a set of high cognitive abilities impairment which have been successfully related to a redox omeostasis imbalance in several psychiatric disorders. Firstly, we aimed to investigate the relationship between executive functioning and some oxidative metabolism parameters in Peripheral Blood Mononuclear Cells (PBMCs) from healthy adult samples. The Brown Attention-Deficit Disorder Scales were administered to assess five specific facets of executive functioning. Total superoxide anion production, Super Oxide Dismutase (SOD), Catalase (CAT), Glutathione Reductase (GR) and Glutathione Peroxidase (GPx) activities were evaluated on proteins extracted from the PBMCs. We found significant positive correlations between superoxide anion production and the total score of the 'Brown' Scale and some of its clusters. The GPx and CAT activities were negatively associated with the total score and some clusters. In a linear regression analysis, these biological variables were indicated as the most salient predictors of the total score, explaining the 24% variance (adjusted $R(2) = 0.24$, ANOVA, $p < .001$). This study provides novel evidence that Executive Functions have underpinnings in the oxidative metabolism, as ascertained in healthy subjects.

1. Introduction

The Executive Function (EF) construction refers to a range of neuropsychological abilities that include inhibitory functions, working memory, and cognitive flexibility, on the basis of which reasoning and problem solving are developed (Lehto et al., 2003; Collins and Koechlin, 2012; Lunt et al., 2012). Executive Function activities are associated with the brain's frontal lobes, and dysfunctions in these areas are related to social, psychological and cognitive functioning. These dysfunctions are also involved in many psychiatric disorders that include the disruption of some aspects of cognition, such as addiction, anxiety disorders, depression, schizophrenia, and primarily, Attention Deficit Hyperactivity Disorder (ADHD) (Elliott et al., 1996; Austin et al., 2001; Gohier et al., 2009).

The majority of EF studies have focused on imaging and genetics. Moreover, some studies adopting a psychobiological approach have suggested that there could be significant correlations between biological factors and certain facets of EFs. It was suggested that an imbalance between antioxidant and pro-oxidant systems may be related to the executive dysfunction.

Under normal physiological conditions, a balance is maintained between both the oxidative and antioxidant systems. Super Oxide Dismutase (SOD), Catalase (CAT), Glutathione Reductase (GR) and Glutathione Peroxidase (GPx), play a key role in the antioxidant network and in the activities of these enzymes which protect cells from a harmful excess of Reactive Oxygen Species (ROS), with superoxide anion leading ($O_2^{\bullet -}$) (Berg et al., 2004).

Studies suggesting a link between EFs and alterations in systemic redox homeostasis are very heterogeneous. The EVA study investigated EFs as a part of a more general cognitive function in an elderly population. The authors highlighted a significant association between poor cognitive performance in two tests assessing visual attention and logical reasoning as well as blood carotenoid levels. Other studies are mainly referred to pathological or conditioning work and environmental settings. As stated by Martínez-Cengotitabengoa et al. (2012), EFs were positively associated with the plasma level of the antioxidant Glutathione (GSH) in schizophrenic patients. In a population of obstructive sleep apnea patients, EFs showed a positive correlation with the

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Super Oxide Dismutase (SOD) protein expression (Sales et al., 2013), and in a 2-year longitudinal study, EFs decline was strongly linked to higher oxidative stress in children undergoing chemotherapy for acute lymphoblastic leukaemia. Finally, the impairment of the working memory was successfully related to a higher total oxidant status and a lower total antioxidant capacity in healthy shift-workers. Whereas, it was suggested that shift-work influences health, performance, activity, and social relationships, as well as being associated with a higher release of pro-inflammatory cytokine Interleukin-1β (Reinhardt et al., 2012).

It should be noted that the association between such forms of executive impairment and redox imbalance is complicated by numerous comorbidities, including aging, body mass index, hypoxemia, daytime somnolence, and above all, inflammatory processes. Inflammatory mediators, such as cytokines, could tip the redox balance into a pro-oxidant state. Hence, it was imperative to investigate if there is an association between EFs and the systemic level of pro-oxidant and anti-oxidant variables in healthy subjects.

Executive processes are strictly linked to emotional functioning and everyday behaviour (Kochanska et al., 2000; Espy et al., 2011; Blair, 2002; Schoemaker et al., 2012). Individuals with impaired EFs show chronic difficulties in managing emotion and frustration, regulating action, focusing on and shifting focus away from tasks, from planning, and from sustained effort over a long period (Damasio et al., 1994). Furthermore, few studies have used EF rating scales, of which the majority utilized the structured neuropsychological laboratory-based tests which had little ecological validity (Thorell, 2007; Miller and Hinshaw, 2010; Miller et al., 2012). It has been shown that EF ratings are better predictors of life impairment in adult ADHD samples (Barkley and Fischer, 2011). However, to date, no studies have evaluated EFs through a psycho-biological approach, focusing on healthy subjects.

The aim of this study is to investigate if certain facets of executive functioning are related to the production of $O_2^{\bullet -}$, and of antioxidant enzymatic activities in psychiatrically healthy samples. The assessment of EFs was performed using a psychometric test. Given the impossibility of taking protein samples from the relevant areas of the brain in healthy subjects, the $O_2^{\bullet -}$ and the antioxidant enzymatic activities needed to be evaluated from peripheral blood mononuclear cells from healthy donors. Because these cells form the main source of ROS, we used them to indirectly evaluate the biological factors underpinning EFs.

2. Materials and methods

2.1. Participants

The participants were an opportune sample of 72 undergraduate students, ranging from 20 to 28 years of age ($M = 23.67$, $SD = 2.92$): 32 men (26.00 ± 4.62) and 40 women (23.28 ± 2.35), recruited from the University of Chieti-Pescara, in the Department of Psychological, Health and Territorial Sciences. All participants gave written informed consent before starting the experiment. The study was complied with the APA ethical standards in the treatment of human samples.

All participants were in good physical health, none of them were on medication, and no one had a history of psychiatric or somatic diseases. The average BMI based on all participants was within the normal range ($M = 22.00$, $SD = 3.73$). The C-Reactive Protein (CRP) serum level was measured as a non-specific marker for inflammation and was utilized as an exclusion criterion. The students recruited, did not show CRP levels >5 mg/L at the time of blood collection and therefore nobody was excluded (Ablij and Meinders, 2002; Biasucci, 2004).

Students who smoke were excluded from the study, because smoking is a strong pro-oxidant and it has been shown to correlate with both the SOD and the Gpx activity (Naga Sirisha and Manohar, 2013).

The participants were invited not to consume alcohol one week (1) before the sample collection in order to avoid any effects on the $O_2^{\bullet -}$ production and on the anti-oxidant enzymatic activities (Benson and Scholey, 2014).

2.2. Psychological measures

In order to assess the EFs functioning, all subjects underwent a psychological assessment via the adult version of Brown's Attention-Deficit Disorder Scales (Brown ADD Scales) (Brown, 1996). The Brown ADD Scales evaluate five symptom domains including Organizing Work (OW), Managing Affective Interference (MAI), Sustaining Energy and Effort (SEE), Working Memory (WM) and Inattention (I). The scale focuses primarily on inattention (Heilingenstein and Keeling, 1995).

The Brown ADD Scales quantifies the EF deficit in daily life, using a 40-item "Likert" scale ranging from 0 (=never) to 3 (=almost daily). The items aim to investigate various issues as a measure of functioning and behaviour in the past six (6) months. Answers are entered on a score sheet; of which five subscales are combined into a total score. The "T" scores are then obtained from the score sheet for each cluster and from the total. High scores indicate greater EF impairment (Taylor et al., 2011).

2.3. Biological measurements

2.3.1. Sample collection

At the same time as the Brown ADD scale was used, venous blood was also collected by phlebotomy into EDTA vacutainers (6 mL K_2EDTA, Becton Dickinson, Franklin Lakes, NJ, USA) and processed within 2 h of procurement. The PBMCs were isolated from the blood of the students by density-gradient centrifugation using Ficoll/Hypaque System (Pharmacia, Piscataway, NJ, USA). Protein extractions were performed as previously described (Pesce et al., 2014).

2.3.2. Quantization of intracellular superoxide production using a colorimetric NBT assay

The Nitro Blue Tetrazolium (NBT) (Sigma–Aldrich) assay was performed according to the method described by Rook et al. (1985) and was used to evaluate the $O_2^{\bullet -}$ production. Briefly, after each treatment, cells were incubated with 0.1 mg/ml NBT in prefiltered culture medium for three (3) hours at 37 °C; and were further washed three (3) times with methanol. The amount of NBT-formazan produced, is an indicator of the intracellular production of the $O_2^{\bullet -}$ and can be determined spectrophotometrically (SpectraMaxH 190, Molecular Devices) at 630 nm after the solubilization of crystals in 200 ml of KOH 2M/DMSO solution.

2.3.3. Cu, Zn-superoxide dismutase (SOD) activity

The SOD activity was determined as described by Sun and Zigman (1978). The assay mixture contained 50 mM of sodium carbonate buffer, pH 10, epinephrine 0.1 mM (Sigma), and protein extract (10 μg) to a final volume of 2.5 ml. The inhibitory effect of the SOD on the autoxidation of epinephrine, using 1.25 mM KCN to discriminate the CN^- insensitive MnSOD from the CN^- sensitive Cu, ZnSOD, was assayed spectrophotometrically at 480 nm at 25 °C. The percentage inhibition values were converted into activities using purified Cu, Zu bovine SOD as the standard (Sigma).