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Association between Salivary Alpha-Amylase and Executive Functioning in Healthy Children

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Abstract. The main aim of this study was to confirm the relationship between executive performance and salivary alpha-amylase (SAA) activity in a sample of 64 healthy children (39 boys), and compare it to the association of SAA output and salivary flow rate (SFR). Executive functioning was assessed via *fluency, trail-making, rings* and *inhibition* tasks from the Batería de Evaluación Neuropsicológica de la Función Ejecutiva en Niños [Battery of Neuropsychological Assessment for Executive Function in Children] (ENFEN), merged into an ENFEN total score. SAA activity, output, and SFR were measured at baseline, one minute before, and one minute after the end of a neuropsychological testing session. Our results confirmed a direct, linear and significant association between SAA activity and executive functioning, $r(64) = .351$, $p < .05$, and extended it to SAA output, $r(64) = .431$, $p < .05$. The mean level of SAA output was the best predictor of executive functioning ($\beta = .431$, $p < .05$) and explained 18.2 % of the variance in ENFEN total score. In sum, and compared to SAA activity, measuring SAA output may be a more precise and indirect marker to assess executive functioning in children.

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Executive functions are relatively complex high-level cognitive processes that emerge during childhood and reach a complete development at older ages (Ardila, 2013). In recent years, different studies have shown how chronic exposure to mild daily stressors can affect the development of these cognitive functions, especially, in socioeconomically deprived families (Berry et al., 2014; Berry, Blair, Willoughby, Granger, & Family Life Project Key Investigators, 2012; Blair, 2010; Blair, Ursache, Greenberg, Vernon-Feagans, & Family Life Project Key Investigators, 2015). Poverty-related adversities include exposure to an increased number of negative biological, psychological, familial and social stimuli (Blair & Raver, 2014). In this way, mild daily psychological stressors on child populations have been identified as noxious and toxic factors for developing brains (Blair & Raver, 2014; Willemen, Koot, Ferdinand, Goossens, & Schuengel, 2008). These findings allow us to build a conceptual bridge to better understand how and why stress can increase child and adolescent psychopathology. These complex cognitive functions, which can be objectively and serially assessed through neuropsychological tasks, serve to effectively regulate cognitive (cold executive functions) and emotional (hot executive functions) processes that form the basis of children's self-regulated behavior (Ardila, 2013; Zelazo & Müller, 2002). A deviation in the development of these cognitive abilities can easily facilitate the maladaptive processes of internalizing problems (due to the inability to manage emotional difficulties) or externalizing behavior (due to difficulties with inhibition and social reinforcement of violent and aggressive behavior). Observing these maladaptive processes in executive functioning can help us simultaneously explain and understand the origins of low academic achievement and some learning difficulties within child populations (Berry et al., 2012; Blair & Raver, 2014; Blair et al, 2015). In this respect, it is well-known that familial (e.g. parenting styles) and environmental (e.g. noise) variables have a constant modulatory effect on executive functions (Berthelsen, Hayes, White, & Williams, 2017; Blair & Raver, 2016).

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Understanding the dynamic process of how psychological stress can impair healthy cognitive development requires a conceptualization according to the diathesis-stress perspective of developmental psychopathology (Sibille & French, 2013). From this point of view, genetic background, gender, neuroendocrine changes, and environmental factors interact in different ways that can lead to early psychopathological and cognitive disorders in children (Blair & Raver, 2014). Among these variables, changes in the two main branches of the physiological stress system associated with developmental cognitive deviations have been the most widely studied (Sapolsky, 2000). When confronting stressors, a child's brain organizes two different but coordinated responses. First, the locus coeruleus-noradrenaline (LC-NE) system, a critical brain system implicated in the regulation of arousal and cognitive flexibility (Aston-Jones & Cohen, 2005; Ramos & Arnsten, 2007; Robbins & Arnsten, 2009), triggers a rapid flight or fight response by activating the autonomous nervous system (ANS), which in turn releases adrenaline and noradrenaline from the adrenal medulla into the blood stream, (Stavrou et al., 2017) and, at the same time, it releases noradrenaline in large regions of the forebrain. In parallel, but with a small delay in its response, the hypothalamic centers organize the HPA-axis response, which ends with the release of cortisol (Nicolaides, Kyratzi, Lamprokostopoulou, Chrousos, & Charmandari, 2015). Both responses are coordinated, but it is believed that they have different, although complementary physiological purposes (Sapolsky, 2000). In studies on child stress, the LC-NE system responses can be monitored through the non-invasive measurement of salivary alpha-amylase (SAA) activity and output (Rohleder & Nater, 2009). SAA activity (U/ml) is a measure of the enzymatic activity of this enzyme in saliva. Secretion of proteins from acinar cells of major salivary glands (like is oral SAA) is mainly under sympathetic control (Rohleder & Nater, 2009) although is known that the co-activation of the parasympathetic branch of ANS can collaborate and increase the release of these salivary proteins in saliva. Salivary flow rate (SFR) is a measure of saliva production over time (ml/min), and in humans is under the exclusive control of the parasympathetic branch of the ANS (Ekström et al., 2009; Nagy et al., 2015; Rohleder & Nater, 2009). Finally, SAA output (U/min) measures the secretion of this enzyme in saliva over time taking into account the changes in salivary flow rate (SAA activity/SFR). It is believed for this reason that this other alternative measure of SAA can integrate the changes of SFR under stress conditions (Arhakis, Karagiannis, & Kalfas, 2013), and so it reduces the possible role of SFR as a physiological confounder. Hence, SAA activity, output and SFR let us to obtain an easy and non-invasive measure of the differential contribution of both branches of the ANS to the cognitive processes under study. Alternatively, HPA-axis activity can be monitored non-invasively through analysis of the free salivary levels of hormone cortisol (Kirschbaum & Hellhammer, 1989). Although many child studies have used salivary cortisol to examine the development of executive functions, studies using SAA activity and output at child ages are still scarce (Nater & Rohleder, 2009).

A detailed review of the few child studies that have employed SAA activity as a biological marker shows how the level of this oral enzyme has been consistently associated with better executive functioning in healthy, rural and low-income samples. Having said that, a full picture of this association only emerges when it is analyzed and combined with the assessment of free salivary cortisol levels. In this way, in 2012, Berry et al. reported that higher basal morning SAA activity levels and moderate levels of cortisol at ages 7, 15 and 24 months predicted better executive functioning (measured as a latent factor using a neuropsychological battery composed of different tests assessing working memory, inhibitory control, and attention shifting) than they did at the age of five. Interestingly, executive functioning and these physiological correlates mediated the academic achievements (measured by the *Woodcock-Johnson Tests of Achievement III applied problems*, quantitative concepts, and letter-word identification subtests) of the participants. In the same vein, Blair and Raver (2014) reported that the *Tools of the Mind* program (Bodrova & Leong, 2007), designed to improve executive functioning (measured in this study through a composite score of the *hearts and flowers task*, the *flanker with reverse flanker task*, and the *NIH toolbox version of the dimensional change card sort task*), also enhanced performance in reasoning ability, vocabulary and mathematics (measured using the *applied problems* and *letter-word* subtests from the *Woodcock-Johnson Tests of Achievement III*) in a cohort of 759 children. In the former study, after *Tools of the Mind* had been applied, SAA activity increased following the sessions. Finally, in a recent study from the same project (i.e. the Family Life Project), the researchers defined the scope of their previous findings. According to their new results, the ability of SAA, cortisol, executive functions performance, and the effortful control temperamental trait to predict better academic achievement was restricted to mathematics abilities and not applicable to reading (Blair et al., 2015). Thus, SAA activity may be considered a new and useful tool for non-invasive monitoring of executive functioning (measured in these previous studies as a composite score of three inhibitory control tasks, two different working memory tasks and an attention shifting task). Nonetheless, given the dearth of published results, additional studies are needed to

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confirm the results of these works in other healthy and clinical samples of children.

Proceeding from these previous results, our concrete aims in this study were two as follows:

(1) To confirm that a better overall executive functioning (measured using the total score of the Spanish *Batería de Evaluación Neuropsicológica de la Función Ejecutiva en niños*, ENFEN) is associated with increased mean levels of SAA activity in a healthy, middle class, urban, community sample composed by 39 boys and 25 girls aged 7 to 12.
(2) To extend these findings by comparing for first time, the usefulness of SAA output measurements (a measurement of SAA never before used to examine this issue) with the most common measurement being its enzymatic activity (Berry et al., 2012; Blair & Raver, 2014; Blair et al., 2015; Rohleder & Nater, 2009). In this sense, we also hope a direct association among SAA output and executive functioning.

Method

Participants

Sixty-four participants from two different schools formed the sample for this study. The participants were aged 7 to 12 years (39 boys) and had good general health. The exclusion criteria included the presence of neurological, cardiovascular, or immunological diseases and any other psychological developmental disorder that could interfere with the salivary analyses (this last information was reported by the family and psychologists from each school center). The level of sexual development could not be assessed for the girls or boys, so other indices of development (i.e. BMI and age) were substituted for them. The study protocol was reviewed and approved by the *Comité de Ética de la Universidad de Málaga* (CEUMA). The study was designed and conducted according to the principles set forth in the *Declaration of Helsinki*. In each session, each participant was asked to provide informed consent, and before the child's participation in the study, every family (mother or father) was informed in a meeting-session about the protocol of the study. They also have to sign the consent form. Table 1 shows the descriptive statistics (mean ± *SD*, range and *n*) of age, body mass index (BMI), family income per month, and education of the participants' parents in our healthy, middle class, urban, community sample.

Procedure

At an initial meeting in each school, every family was completely informed of this study's protocols and received detailed information on both the neuropsychological testing procedures and the collection of saliva samples. The neuropsychological assessments were conducted during the mornings on school days (always between 9:30 and 12:30). In each session, small groups of two to four participants were guided to a quiet room inside the school where trained personnel conducted the assessments. Saliva samples were taken at baseline (collected before the test, after a 10 to 15-minute wait outside the test room), in the pre-test moment (before the start the neuropsychological assessment) and the post-test moment (just after the end of the last task). We repeated the same strategy with three different sets of neuropsychological tests, although only data for the ENFEN battery are shown here. The order in which the tests and batteries were applied was counterbalanced. The order of testing, the time taken to complete the battery tasks, gender, age, and BMI were included in our statistical analyses as covariates.

Instruments

The *Batería de Evaluación Neuropsicológica de la Función Ejecutiva en Niños* (ENFEN) is a Spanish battery of tests designed to assess executive function (Portellano, 2009). This tool evaluates the main components of executive functions from age 6 to 12. The battery includes five main tasks: *Motor Inhibition* (not used in this study), *Phonological* (PF) and *Semantic Fluency* (SF), a *Trail-Making Test in black & white* (TMBW) *and color* (TMC) versions, a modification of the *Hanoi Towers Test* called *Rings* (R), and an *Inhibition* (I) task derived from the *Stroop Test*. The instrument has been validated and scored for Spain's child population and shows good psychometric properties. In this study, for the entire battery the Cronbach's alpha coefficient was 0.572; however, when the I task score was excluded it increased to 0.714 (see the discussion section for more details on this decision). Table 2 shows the performance for each scale as a direct score in this battery.

The PF and SF tasks inform us of each participant's ability to produce language under time pressure according to a phonetic or semantic rule, although this has also been described as an indirect measurement of working memory. According to diverse neuroimaging studies, these tasks involve activity from diverse areas of the brain network responsible for language production (Ocklenburg, Beste, Arning, Peterburs, & Güntürkün, 2014; Skeide & Friederici, 2016). The TMBW and TMC provide information on various complex cognitive functions of the participants. These scores cover different aspects of executive functioning such as flexibility, thinking strategies, inhibition, working memory, prospective memory, executive attention, visuospatial abilities, and fine motor skills (Portellano, 2009). According to previous studies conducted on healthy

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Table 1. *Descriptive Statistics ($\bar{X}$ ± SD, Range and n) of Age, Body Mass Index (BMI), Family Income per Month and Parental Education (for Available Data).*

Age	BMI	Family Income/ month euros	Parental education Fathers (number of cases)	Parental education Mothers (number of cases)
$\bar{X}$ ± *SD* (range)	$\bar{X}$ ± *SD* (range)	$\bar{X}$ ± *SD* (range)		
n	*n*	*n*	*n*	*n*
9.92 ± 1.45 (7.3 – 12.3)	14.48 ± 3.63 (8.89 – 28.71)	2,433.88 ± 926.89 (600 – 5,300)	No studies: 1	No studies: 0
64	63	50	Less than primary school studies: 3 Primary school studies: 22 Secondary School studies: 15 University studies: 5 Master´s degree: 15 *n* = 61	Less than primary school studies: 0 Primary school studies: 15 Secondary School studies: 18 University studies: 11 Master´s degree: 16 *n* = 60

Note: BMI = Body Mass Index

Table 2. *Descriptive Statistics ($\bar{X}$ ± SD, Range) of Direct Scores in ENFEN Battery for the Complete Sample (n = 64)*

Scale	PF	SF	TBW	TC	R	I	ENFEN-TS
$\bar{X}$ ± SD (range)	8.56 ± 3.74 (1–20)	15.42 ± 4.50 (6–28)	23.36 ± 7.13 (10.16–45.79)	13.74 ± 5.11 (5.88–29)	7.61 ± 0.66 (5.63–8.86)	73.31 ± 21.45 (18–126)	13.74 ± 3.23 (6.77–23.22)

Note: PF = Direct score in Phonologic Fluency; SF = Direct score in Semantic Fluency; TBW = Direct score in Trail-Making Test Black & White version; TC = Direct score in Trail-Making Test Color version; R = Direct score in Rings; I = Direct score in Inhibition; ENFEN-TS = Total Score in ENFEN battery.

and pathological child populations, these functions involve the activity of diverse regions of the prefrontal cortex in conjunction with other posterior regions of the neocortex (Ardila, 2013; Noordermeer, Luman, & Oosterlaan, 2016). Correspondingly, the R scores represent the result of a complex mix of different executive functions in which planning, flexibility, prospective memory, abstraction, fine motor skills, and working memory work together to solve each of the 15 assignments in this task. Finally, I is a relatively pure measure of the cognitive inhibition processes directed by the dorsolateral and orbitofrontal areas of the prefrontal cortex, as diverse studies have reported (Congdon & Canli, 2005). Although this version of the Stroop Test measures cognitive inhibition, posterior attention and flexibility are also cognitive functions engaged in the performance of this task.

The authors of this test emphasize that after factorial analysis, a unique factor emerged, explaining 49% of the variance and showing the one-dimensional nature of all of the tasks in this battery. For this reason, all of the scores from these tasks (except I, which was removed due to the low Cronbach's alpha coefficient obtained for the complete neuropsychological battery) were averaged to create an unique total score for executive functioning what it has been denominated ENFEN total score (ENFEN-TS). We discuss each specific score and its association with SAA in the Results section. A more detailed description of the developmental changes observed in these test scores can be found in the book on this battery (Portellano, 2009).

Salivary analyses

Saliva samples were obtained using the passive droll method. In each session, the participants were instructed to accumulate saliva in their mouth and after two minutes provide the first sample with the remaining samples provided after one minute. After the end of testing, the saliva samples were immediately placed into a small portable fridge containing ice to protect them from temperature change. Later, the samples were frozen in our laboratory at -20°C. Salivary alpha-amylase activity and output assays were realized through an enzymatic colorimetric assay

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(see Sánchez-Navarro, Maldonado, Martínez-Selva, Enguix, & Ortiz, 2012 for more details). The SAA activity, output and SFR levels are described in Table 3. The intra- and inter- CV were below 10% in our measurements (data not provided). In the case of a violation of normality, the salivary data (SAA activity, output and SFR) were square-root transformed.

Statistical analyses

Initially, our statistical analyses aimed to describe the socioeconomic, demographic and anthrometric characteristics of our participants (Table 1). The order of testing, time used to complete the battery tasks, gender, age, and BMI were included as possible covariates of salivary measurements using repeated-measure ANOVA tests. In the case of the Rings scores we inverted it according to the next formula for each participant: (1000–Ring score) / 100. After these steps, based on our statistical analysis plan, we first assessed the association (moment-product Pearson's correlation coefficients) between the mean of the salivary measures and the ENFEN scores, and then determined through linear regression analyses (using the step-wise method) the best salivary predictor of ENFEN-TS and the rest of ENFEN scores. For all analyses, *p*-values of < .05 were considered significant, but correlational analyses were performed with a Bonferroni correction to control the overall level of significance. The data were analyzed using the PASW 18 version. Unless otherwise indicated, all results shown in the tables and figure are mean ± *SD* (obtained from untransformed data) to facilitate comparison with previous and future studies.

Results

The socioeconomic, demographic and anthrometric characteristics of our participants are showed in Table 1. The descriptive statistics for the ENFEN´s direct scores are shown in Table 2. And in Table 3 describes the baseline, pre-test, post-test levels of SAA activity, SAA output and SFR (mean ± *SD* and range) during the testing session. The repeated-measures ANOVA did not show a time effect for SAA activity along the assessment session, $F(2, 126) = 2.036$, $p = .146$, $\eta^2 = .365$, but it did for SAA output, $F(2, 126) = 2.054$, $p = .025$, $\eta^2 = .674$. BMI, gender, age, order of testing and time spent in testing were excluded as possible covariates due to their non-significant contribution to SAA activity and output patterns in our statistical analyses. In our posterior analyses, we used mean levels of SAA activity, output and SFR to better capture the sustained effect of the cognitive performance of the ENFEN´s tasks on SAA and SFR.

Our statistical correlational analyses (Table 4) pointed out a statistically significant association between SAA activity/output and ENFEN-TS scores. SAA activity/output also showed a statistically significant association with TMBW scores. These linear relationships were direct in all cases, indicating that higher levels of SAA activity and/or output were observed among the participants with more efficient performance in executive functions (Table 4). The associations between the mean SAA output and ENFEN-TS scores were as strong as those observed for mean SAA activity according with these analyses.

In the second step of our statistical analyses, we used linear regression analyses to determine the best salivary predictor of ENFEN-TS. According to our statistical analysis, ENFEN-TS was best predicted by the mean SAA output levels ($\beta = .431$, $p < .05$), which explained 18.2 % of the variance. To analyze the possible confounding effect of multicollinearity in our study, we calculated two separated models including only one of both predictors (SAA activity or SAA output) and SFR. According with the results of this analytical strategy, the model with a higher adjusted R-squared coefficient to predict the ENFEN total score was the model in which we introduced as unique predictor SAA output. In the same vein, SAA output was also the best predictor of PF ($\beta = .257$, $p < .05$), TMBW ($\beta = .436$, $p < .05$) and R ($\beta = .300$, $p < .05$) scores. Finally, TMC was best predicted by the mean SAA activity levels ($\beta = .345$, $p < .05$).

Table 3. *Descriptive Statistics ($\bar{X}$ ± SD and Range of Non-Transformated Data) of Salivary Measures (n = 64)*

SAA activity (U/ml)	Baseline	Pretest	Posttest	Mean levels
$\bar{X}$ ± *SD* (range)	128.26 ± 88.64 (16–411.33)	149.29 ± 103.93 (15–533)	145.37 ± 106.38 (23–621.83)	140.97 ± 89.35 (19.83–522.05)
SAA output (U/min)	Baseline	Pretest	Posttest	Mean levels
$\bar{X}$ ± *SD* (range)	85.91 ± 101.06 (4.09–429.25)	100.72 ± 112.73 (1–580.97)	90.78 ± 77.09 (1.02–335.79)	92.47 ± 85.87 (2.04–448.18)
SFR (ml/min)	Baseline	Pretest	Posttest	Mean levels
$\bar{X}$ ± *SD* (range)	0.62 ± 0.46 (0.11–2.10)	0.65 ± 0.40 (0.01–1.95)	0.66 ± 0.41 (0.01–1.97)	0.64 ± 0.37 (0.06–1.78)

Note: SAA = Salivary Alpha-Amylase; SFR = Salivary Flow Rate.

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Table 4. *Bivariate Correlations (Pearson`s Correlation Coefficient and Exact p Value) between Direct Scores in the ENFEN Battery and Salivary Measures (n = 64)*

	PF	SF	TBW	TC	R	ENFEN-TS
$\bar{X}$ SAA Activity (U/ml)	.257	.102	.413[a]	.351	.276	.393[a]
	.020	.210	.000	.002	.014	.001
$\bar{X}$ SAA Output (U/ml)	.300	.045	.482[a]	.339	.249	.412[a]
	.008	.363	.000	.003	.024	.000
$\bar{X}$ SFR (ml/min)	.146	–.0001	.256	.053	–.123	.168
	.125	.496	.021	.340	.167	.092

Note: [a]Using Bonferroni corrections (significance level set at α(.05)/18 = .002) and using unilateral contrasts.

PF = Direct score in Phonologic Fluency; SF = Direct score in Semantic Fluency; TBW = Direct score in Trail-Making Test black & white version; TC = Direct score in Trail-Making Test Color version; R = Direct score in Rings; I = Direct score in Inhibition; ENFEN-TS = Total Score in ENFEN battery; SAA = Salivary Alpha-Amylase; SFR = Salivary Flow Rate.

Discussion

The main findings of this study have confirmed that better executive functioning is associated with levels of SAA activity and/or output during controlled neuropsychological assessment. These findings are consistent with the results of Berry et al. (2012), Blair and Raver (2014) and Blair et al. (2015), although in this study we did not obtain a parallel measurement of cortisol and we used a different selection of tasks compared to the previous research (Willoughby, Blair, Wirth, & Greenberg, 2010) conducted in healthy, low-income, rural, community samples. In our study, SAA activity and output allowed us to predict better performance in a composite score of different but related tasks measuring divergent aspects of executive functioning in healthy, middle class, urban, community sample composed by boys and girls aged 7 to 12 under good conditions of reliability and a high level of ecological validity. Thus, this is the first study to use SAA output measurements to examine their association with cognitive performance in children.

Our results show for the first time how both mean SAA measurements (SAA activity and output) exhibited a relationship predicting the ENFEN total score. Our regression analysis demonstrated that the SAA output measurement was a better marker than SAA activity for some of the ENFEN tasks (i.e. TMBW) and the total score in this battery. SAA output is considered a better physiological measure of the biological process implicated in the protein release in saliva under stressful cognitive and social conditions. Future studies using SAA output as a research tool should examine the utility of this other parameter in depth. In sum, our results suggest that increased levels of peripheral NE and ANS tone (sympathetic and parasympathetic) correlate with better executive functioning performance, based on the association between SAA and these ANS activities (Ditzen, Ehlert, & Nater, 2014; Kuebler et al., 2014; Nater & Rohleder, 2009; Schumacher, Kirschbaum, Fydrich, & Ströhle, 2013; Warren, van den Brink, Nieuwenhuis, & Bosch, 2017). This result is consistent with the recently proposed role of the LC-NE system as a brain system critical to executive functioning and cognitive flexibility (Aston-Jones & Cohen, 2005; Ramos & Arnsten, 2007; Robbins & Arnsten, 2009).

Finally, although it is beyond the scope of this study, we can speculate on the nature of the association between SAA and executive functioning. SAA activity has shown a statistically significant correlation with ANS activation in previous child studies (Nater & Rohleder, 2009; Schumacher et al., 2013). This oral enzyme has also shown an association with peripheral noradrenaline (NE) levels in healthy students confronted with psychosocial and cognitive stressors (Ditzen et al., 2014) that increased after the administration of the NE transporter blocker atomoxetine (Warren et al., 2017) and NE infusions (Kuebler et al., 2014), but decreased after beta blockade and the administration of propranolol (van Stegeren, Rohleder, Everaerd, & Wolf, 2006). Together, these results suggest the potential use of SAA as an indirect marker of ANS activation and the peripheral activity of NE governed by the LC-NE system.

However, we might also suggest an alternative hypothetical association between SAA and the central noradrenergic activity directed by the LC-NE system in accordance with the hypothesis originally suggested by Ehlert, Erni, Hebisch, and Nater (2006). Unfortunately, it has been difficult to determine this association due to technical problems and difficulties involved with analyzing CSF in child samples. Nevertheless, in the future, the use of transcutaneous vagus nerve stimulation (tVNS), a non-invasive neurological manipulation that increases central NE levels, may help us with this objective (van Leusden, Sellaro, & Colzato, 2015). Although it was only a preliminary result, Weymar et al. (2017) found a statistically significant increase in

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SAA activity in association with larger P300 amplitudes related to targets in oddball tasks after using tVNS in 20 healthy participants. In the same vein, the percentage of change in SAA activity has been associated with change in pupil dilatation responses (a physiological response under the exclusive control of the sympathetic branch of the ANS; Nielsen & Mather, 2016). The LC-NE system function of our brain is key to understanding successful executive and flexible functioning, and SAA might represent a methodological tool, remarkably useful to partially examining central LC-NE activity in a non-invasive and repeated manner in healthy clinical child populations.

Unfortunately, our study exhibited some weaknesses that limit the generalization of our findings. First, it had a small sample of only 64 participants that, nevertheless, let us to confirm the association among SAA and executive functioning. Second, the lower Cronbach's alpha coefficient initially obtained from all of the ENFEN scales (PF, SF, TMBW, TMC, R and I) in this study led us to exclude the I scores. This low initial Cronbach's alpha value would be justified by the order of measurements in this task (the last one) and for the specific cognitive skills required (cognitive inhibition) by a group of boys and girls with a wide range of ages. Third, although we included participants of both genders, we were unable to create an adequate sample to assess their differences in this respect. Gender differences in the developmental profile of executive functions have been observed from early childhood ages by different research groups (Blair & Raver, 2014; Blair et al., 2015). Fourth, the participants enrolled in this study came from only two different schools, which prevented us from obtaining a representative sample from each SES segment in our city, both rural and urban (Ursache & Noble, 2016). Finally, in this study we did not obtain any psychological measure of stress, arousal or valence related to the feelings of the participants during their assessment sessions (Sánchez-Navarro et al., 2012). In the same vein, we could not include the measurement of salivary cortisol levels in our participants (Berry et al., 2012). In future studies, the inclusion of the measurement of this steroid may help us understand in depth the precise nature of the association between SAA and executive functioning.

In conclusion, the main findings of our study confirmed and extended prior findings on the direct and linear association between executive functioning and SAA activity levels, occurring in the same assessment session, in a sample of healthy boys and girls, aged 7 to 12, from an urban middle class sample. As a novel finding of our study, we observed that mean SAA output levels were a better marker of this association compared to mean SAA activity levels.

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