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Physical activity effects on the individual alpha peak frequency of older adults with and without genetic risk factors for Alzheimer's Disease: A MEG study

Jaisalmer de Frutos-Lucas [a,b,*,1], David López-Sanz [a,c,1], Pilar Zuluaga [d], Inmaculada Concepcion Rodríguez-Rojo [a,c], Raúl Luna [e], María Eugenia López [a,c], María Luisa Delgado-Losada [c], Alberto Marcos [f], Ana Barabash [g], Ramón López-Higes [c], Fernando Maestú [a,c,h], Alberto Fernández [a,i]

[a] Laboratory of Cognitive and Computational Neuroscience (UCM-UPM), Center for Biomedical Technology, Pozuelo de Alarcón, Spain
[b] Biological and Health Psychology Department, Universidad Autonoma de Madrid, Madrid, Spain
[c] Department of Experimental Psychology, Universidad Complutense de Madrid, Pozuelo de Alarcon, Spain
[d] Department of Statistics and Operational Research, Medical School, Universidad Complutense de Madrid, Madrid, Spain
[e] Department of Basic Psychology I, Universidad Complutense de Madrid, Madrid, Spain
[f] Departamento de Neurologia, Hospital Clinico San Carlos, Madrid, Spain
[g] Laboratory of Psychoneuroendocrinology and Genetics, Hospital Clinico San Carlos, Instituto de Investigacion Sanitaria del Hospital Clinico San Carlos (IdISSC), Madrid, Spain
[h] Biomedical Image Technologies, Universidad Politecnica de Madrid and CIBER, Madrid, Spain
[i] Department of Legal Medicine, Psychiatry and Pathology, Medical School, Universidad Complutense de Madrid, Spain

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HIGHLIGHTS

- The individual alpha peak frequency (IAPF) is sensitive to AD pathology.
- Physical activity (PA) positively influences IAPF, but only significantly among APOE ε4 non-carriers.
- Results offer the first MEG evidence on the combined effects of *APOE* genotype and PA.

ABSTRACT

Objective: Since a cure for Alzheimer's Disease (AD) is yet to be discovered, attention has shifted towards prevention. Physical activity (PA) emerged as a notorious lifestyle factor that could influence brain structure and function. The individual alpha peak frequency (IAPF) is a measure that summarizes the spectral content of brain signals and has been proven to be sensitive to both AD pathology and PA interventions. Therefore, our goal was to unravel whether chronic PA modulates IAPF and if *APOE* ε4 carriage moderates this relationship.
Methods: We analyzed 4-minutes of resting-state magnetoencephalographic recordings from 100 healthy elders that provided self-reported measures of PA, and the IAPF was calculated.
Results: We found that IAPF was negatively influenced by age and *APOE* and positively influenced by PA. The effect of PA on IAPF only remained significant for the ε4 non-carriers group.
Conclusions: PA is positively associated to higher IAPF in healthy older adults and could potentially act as a protective factor against cognitive decline. Nevertheless, such effect is non-significant among elders who are more vulnerable to developing AD due to their genetic carriage.
Significance: This investigation offers the first neurophysiological evidences on the combined effects of *APOE* genotype and PA in healthy elders.

Abbreviations: IAPF, individual alpha peak frequency; AD, Alzheimer's Disease; PA, physical activity; MCI, mild cognitive impairment; *APOE*, apolipoprotein E gene; EEG, electroencephalography; MEG, magnetoencephalography; IPAQ, International Physical Activity Questionnaire; MET, Metabolic Equivalent of Task; GLM, general linear model; CPA, categorical physical activity; LMET, *log* transformed Metabolic Equivalent of Task.

* Corresponding author at: Parque Científico y Tecnológico de la Universidad Politecnica de Madrid, Crta. M40, Km. 38, 28223 Pozuelo de Alarcón, Madrid, Spain.
E-mail address: jaisalmer.defrutos@ctb.upm.es (J. de Frutos-Lucas).

[1] These two authors equally contributed to this manuscript and share first authorship.

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1. Introduction

For more than two decades, an enormous effort has been devoted to the search for pharmacological therapies that could palliate the devastating effects of Alzheimer's Disease (AD). Most of these therapies relied on the so-called "amyloid hypothesis" (Hardy and Higgins, 1992), that is considered the leading explanation for the disease. Unfortunately, clinical trials testing agents that interfere with amyloid production failed to improve cognition or to slow down the deterioration process, while produced substantial side effects (Drachman, 2014; Intlekofer and Cotman, 2013). As a consequence, it is crucial to identify alternative strategies that might be able to delay the development of a clinically significant cognitive impairment.

A potential alternative to pharmacological therapies is the intervention in modifiable lifestyle behaviors. Among others (e.g. sustained cognitive activity, dietary and leisure-time habits, etc.), physical activity (PA) emerged as a promising factor that might retard the development of cognitive deterioration (Raz et al., 2005; Sofi et al., 2011; Buchman et al., 2012; Zhu et al., 2015). A sustained PA has been associated with improved cognitive functioning in the aged (Ahlskog et al., 2011; Etgen et al., 2010; Smith et al., 2010). Such improved functioning, especially in the memory domain, goes in line with the fact that older adults involved in regular PA showed greater medial-temporal volumes (Bugg and Head, 2011; Smith et al., 2014; Okonkwo et al., 2014; Carlson et al., 2015; Brinke et al., 2015). Medial-temporal volumetry is not the only biological marker that enables an objective assessment of PA effects. Functional techniques such as fMRI or PET revealed that PA produces significant influences on brain activity, both at resting state and task-induced conditions (for an exhaustive review on the field see Smith et al., 2013).

Comparatively, less information is currently available regarding the effects of acute and chronic PA on neurophysiological measures. Classical EEG studies indicated that older subjects in good aerobic condition exhibited increased alpha activity (Dustman et al., 1994; Dustman et al., 1990), and reduced delta activity (Lardon and Polich, 1996). This evidence is of high relevance, since an increased alpha accompanied by decreased delta activity is the opposite pattern to that observed in AD and mild cognitive impairment (MCI) (Huang et al., 2000). Subsequent research (Crabbe and Dishman, 2004) supported the finding of increase of alpha activity associated with a state of relaxation after PA, although some others reported a decrease of beta activity (Schneider et al., 2009). In accordance with previous research, Fumoto et al. (2010) found variations within the alpha range, with an augment of high alpha activity during and after a session of acute PA (i.e. pedaling exercise). Recently, Gutmann et al. (2015) proposed the calculation of the individual alpha peak frequency (IAPF) as a more comprehensive approach to assess PA effects on neurophysiological measurements, and performed the first study on this field. The IAPF corresponds to the discrete frequency showing the highest power within the alpha range, and can be intuitively interpreted as a proxy that summarizes the spectral content of brain signals. More importantly, IAPF values correlate with cognitive status in healthy aged subjects and are also impaired across several medical conditions other than AD, including autism and stroke (Rodriguez et al., 1999; Garces et al., 2013; Lopez-Sanz et al., 2016; Petrovic et al, 2017; Dickinson et al., 2018). Gutmann et al.'s results demonstrated that an exhaustive exercise protocol significantly increased IAPF values in a sample of young adults.

The above presented investigations tended to show that PA induces significant beneficial effects on cerebral and cognitive functioning, however not all studies are in line with this tendency (see Smith et al., 2013). A plausible explanation for such discrepancy derives from the fact that some additional factors might act as modifiers, exerting a clear influence on the risk of cognitive impairment. Perhaps the best example of this type of factors is the apolipoprotein E gene (*APOE*). The *APOE* gene is located on chromosome 19q13 and in the brain it is mainly expressed in astrocytes. The resultant glycoprotein ApoE is involved in lipid transportation, beta-amyloid clearance, synaptogenesis, neural repair and neuroplasticity among other actions (see Kim et al., 2014; Mahoney-Sanchez et al., 2016, for a comprehensive review on the biological function of ApoE). Nowadays it is well-known that carriers of the ε4 allele have a greater risk of developing AD and more spread neuropathological signs (Modrego, 2006; Habib et al., 2017). Hence, PA effects on brain function may differ according to the *APOE* genotype (Etnier et al., 2015). Although this is a quite logical assumption, until recently little attention had been paid to such an important question within some research domains. Particularly, to the best of our knowledge, no previous electroencephalography (EEG) or magnetoencephalography (MEG) study had assessed the combined effects of PA and *APOE* genotype on spectral measures. Considering this situation, we carried out a MEG study that evaluated the potentially interactive effects of PA and *APOE* genotype (together with some other factors such as educational attainment, age or gender) on the IAPF of a group of healthy elders. Our objective was twofold: first, to determine whether Gutmann et al.'s findings of increased IAPF values associated with physical exercise can be extrapolated to the elderly population; and secondly, to evaluate whether *APOE* genotype is capable to modulate the effects of PA on the IAPF.

2. Methods

2.1. Subjects

One-hundred community dwelling volunteers (69 females) were recruited from the "Centro para Mayores del Distrito de Chamartín", and the "Servicio de Neurología del Hospital Clínico Universitario San Carlos", Madrid, Spain. All of them were right-handed (Oldfield, 1971) and native Spanish speakers. Demographic data are shown in Table 1.

All subjects underwent an exhaustive clinical and neuropsychological assessment, MRI, and genetic analysis (for a detailed description of the protocol utilized by our group see Lopez-Sanz et al., 2016). The exclusion criteria were as follows: 1. Present history of psychiatric disorders or past/present history of neurological disorders (reported during a semi-structured interview, following DSM criteria); 2. Evidence of infection, infarction, focal lesions in a T2-weighted MRI scan within 2 months before MEG acquisition; 3. Evidence of significant hippocampal atrophy in a T1-weighted MRI scan within 2 months before MEG acquisition; 4. A modified Hachinski score equal to 5 or higher; 5. Current alcoholism, chronic use of anxiolytics, neuroleptics, narcotics, anticonvulsants or sedative hypnotics that could affect MEG activity; and 6. An age under 65 years. The study was approved by the Hospital Universitario San Carlos ethics committee and all subjects gave informed consent prior to their participation.

2.2. Physical activity measurement

All participants completed the short version of the International Physical Activity Questionnaire (IPAQ). This tool quantifies self-reported levels of physical exercise in the past seven days. Subjects must provide information about how many days and during how many minutes they engaged in vigorous physical activity, moderate physical activity or walking for more than ten minutes. Levels of physical activity were calculated in Metabolic Equivalent of Task (MET) units. Vigorous activity is allocated 8 METs, moderate

Table 1
Mean values ± standard deviation of the demographics and more relevant characteristics of the sample (N = 100).

Age (mean ± SD)	71.84 ± 4.28
Sex (males)	31
Formal Education (years; median (IQR))	13.00 (12.00)
APOE ε4 non-carriers	80
MMSE score (mean ± SD)	28.54 ± 1.66
Backward Digit Span score (mean ± SD)	5.85 ± 1.93
TMTb score (mean ± SD)	22.70 ± 3.06
IAPF (mean ± SD)	9.44 ± 0.88
MET (median (IQR))	1371.00 (1615.87)
Subjects per levels of physical activity:	
Low	24 (17 carriers)
Moderate	60 (51 carriers)
High	16 (12 carriers)

SD: Standard Deviation; IQR: Interquartile Range; *APOE* ε4: Apolipoprotein E epsilon 4; MMSE: Mini Mental State Examination; TMTb (Trail Making Test part b) IAPF: Individual Alpha Peak Frequency; MET: Metabolic Equivalent of Task.

activity is allocated 4 METs and walking is allocated 3.3 METs. MET scores are multiplied by the number of days per week and minutes per day that subjects developed such activity. The total activity in the three categories was later added up in order to determine a weekly measure of PA. Subsequently, participants were classified into groups of low, moderate or high physical activity. All these measures were obtained following the guidelines (see Table 2) of the IPAQ user's manual (http://www.ipaq.ki.se).

2.3. Educational attainment

Considering previous investigations that highlighted the role of educational attainment as a sensitive estimate of cognitive reserve, this proxy was measured as years of formal education (range 1–20 years), and obtained by questioning participants or caregivers (Lopez et al., 2016).

2.4. APOE genotyping

Genomic DNA was extracted from 10 ml blood samples in ethylenediaminetetraacetic acid. Detection of *APOE* genotype was performed with TaqMan technology using an Applied Biosystems 7900 HT Fast Real Time PCR machine (Applied Biosystems, Foster City, CA). For more information see the genotyping method previously described in Cuesta et al. (2015). As a result, three alleles of the *APOE* gene were obtained: ε2, ε3 and ε4. According to the *APOE* ε4 allele noxious effect, participants were classified as *APOE* ε4 carriers (i.e. ε3ε4) and non-carriers (i.e. ε3ε3) (see Table 1).

Table 2
IPAQ classification criteria.

High	Moderate	Low
Vigorous-intensity activity on at least 3 days and accumulating at least 1500 MET-minutes/week OR 7 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 3000 MET-minutes/week	3 or more days of vigorous activity of at least 20 min per day OR 5 or more days of moderate-intensity activity or walking of at least 30 min per day OR 5 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum of at least 600 MET-min/week	Otherwise

2.5. MEG recordings

Each subject underwent a 4-minutes MEG recording of awake resting state activity while sitting comfortably. The system used was a Vectorview MEG with 306 channels (102 magnetometers and 204 planar gradiometers). Head position information was continuously acquired using 4 coils attached to the participant́s forehead (left and right) and bilateral mastoids to control for head motion. Additionally, we used two vertical electrooculogram electrodes to capture and remove eye blinks and movements. Electromagnetic activity was recorded with 1000 Hz sampling rate and online filtered between [0.1 330] Hz. Afterwards, MEG data were filtered offline using the temporal extension of the signal space separation algorithm implemented in Maxfilter 2.2 software.

2.6. MEG preprocessing and individual alpha peak frequency calculation

We used an automatic procedure implemented in the Fieldtrip package (Oostenveld et al., 2011) to detect jump, muscular and blink artifacts in magnetometers. Artifacts were visually confirmed by a MEG expert. In addition, an independent component analysis based procedure was employed to remove electrocardiographic and electrooculogram components. We extracted segments of 4 consecutive seconds of artifact-free signal from the preprocessed data.

Clean segments were band-pass filtered between 2 and 45 Hz to avoid low frequency noise and network line artifact. Furthermore, 2000 samples (i.e. 2 s) of real data were maintained at both sides of the segments to avoid edge artifacts when filtering. Afterwards power spectrum was estimated for each segment (48.24 ± 8.26 segments in average) using Fieldtrip toolbox. For each of the segments in each sensor, we employed discrete prolate spheroidal sequences as tapers with 0.5 Hz smoothing to estimate power between 2 and 45 Hz in 0.5 Hz frequency steps. The resulting absolute power value obtained for each frequency step was divided by the sum of the individual broad band power spectrum for that sensor, thus resulting in the normalized or relative power spectrum employed in our analysis. The power spectra matrices (102 sensors by 87 frequency steps by *n* clean segments) were finally averaged across segments thus leaving a relative power value for each sensor and frequency. In our study the IAPF value was visually identified over an average of occipital and posterior temporal sensors as the most prominent power in the alpha range (for more information on MEG methods, see Lopez-Sanz et al., 2016). To ensure peak identification reliability, we additionally estimated IAPF by using an automatic algorithm which fits power spectrum to a Gaussian peak with a power law background (as described in Lopez-Sanz et al., 2016). Afterwards, we used an Intraclass Correlation Coefficient to estimate the inter-rater reliability and obtained a value of 0.9 which is considered an excellent agreement between methods. A high reliability of alpha power estimates has been previously reported in the literature (Martín-Buro, Garcés and Maestú, 2016), thus ensuring the stability and soundness of our analyses.

2.7. Statistical analyses

In this study, we analyzed the relationship between IAPF and PA level in healthy aged subjects who were split into two groups, *APOE* ε4 carriers and *APOE* ε4 non-carriers. The characteristics of gender, age, years of formal education, *APOE* ε4 genotype (*APOE*), MET values and PA are shown in Table 1. Kolmogorov-Smirnov-Lilliefors and Shapiro-Wilk tests were used to assess the normality of all variables. Continuous variables were expressed as means and standard deviations (SD) or as medians and interquartile ranges (IQR) in case they violated the normality assumption. Categorical

variables were presented as frequencies (percentages). When the normality assumption was violated, data were normalized using a log transformation. The transformed variables will be henceforth denominated by adding the "L" prefix to the name of the original variable.

A several-step procedure was carried out. Firstly, a series of exploratory analyses were performed in order to select potential candidates that may be included in a general linear model (GLM). Statistical significance of continuous variables with normal distribution was determined using Student's *t*-test or ANOVA (for two or more categories), and the significance of differences between categorical variables was determined using the Chi-square test. Linear regression and Pearson's correlation were used to see the relationship between continuous normally-distributed variables; otherwise Spearman's correlation was utilized. Secondly, it was important to check the balance among groups in terms of relevant variables (covariates) that could modify the effects of the main factor. Finally, a multivariate ANCOVA was used to investigate the effects of PA level on IAF, and the potential modifying influences of the selected relevant covariates. All data were tested to ensure conformity with the assumptions of ANCOVA testing, including normality and homoscedasticity. Bonferroni tests were used for all post-hoc comparisons. We quantified the effect size using partial eta-squared, and also reported the observed power.

Statistical analyses were performed using SPSS 22.0.

3. Results

3.1. Exploratory analysis of factors and covariates

As briefly explained in the Methods section, our protocol includes two variables for the assessment of PA: 1. A categorical variable that classifies subjects in three levels of PA (high, moderate and low, see above), henceforth called "categorical PA" (CPA); and 2. A quantitative variable that refers to MET values. Consequently, our first goal was to determine which variable (CPA or MET) best describes the behavior of the IAPF. MET is a continuous variable but its values are not normally distributed (Kolmogorov-Smirnov-Lilliefors test, K-S = 0.313; $p < 0.001$). Therefore, MET values were log transformed (i.e. LMET) to match a normal distribution (K-S = 0.081; $p = 0.108$). Pearson's test demonstrated a significant correlation between IAPF and LMET (rho = 0.243; $p = 0.020$). Then, we studied the relationship between CPA and LMET by means of one-way ANOVA. As it might be expected, the ANOVA model demonstrated a strongly significant influence of CPA levels on LMET values ($F_{2,97} = 132.006$; $p < 0.001$). In addition, the value of partial eta-squared was 0.733, that is to say, a 73.3% of all the variance in LMET was explainable by the effect of CPA. Finally, we performed an ANCOVA test with LMET and CPA as covariate and fixed factor, respectively, to assess their potential influence on the IAPF. The ANCOVA model revealed a significant effect of CPA levels on the IAPF ($F_{2,96} = 4.061$; $p = 0.020$), but no significant influence of LMET included as a covariate ($F_{1,96} = 0.567$; $p = 0.453$). The adjusted-R^2 of the model was 0.102. Considering these evidences, it seemed reasonable to assume that CPA and LMET are strongly associated and provide very similar or even redundant information, but CPA is a better variable to describe the behavior of the IAPF. Consequently, CPA will be submitted to the final GLM analyses (see Fig. 1). Moreover, the mean power spectrum for each group of CPA are presented in Fig. 2.

Once this preliminary but important question was addressed, we investigated the relationship among the IAPF and several factors. For instance, a statistically significant inverse linear relationship between the IAPF and age (linear regression *t*-test, $t_{98} = 2.207$; $p = 0.030$) was found, indicating that IAPF values decreased with age. The mean of the IAPF was significantly higher (Student's *t*-test, $t_{98} = 2.385$; $p = 0.019$) for *APOE* non-carriers than for *APOE* carriers. Females had a statistically significantly higher IAPF mean than males (Student's *t*-test, $t_{98} = 2.866$; $p = 0.005$). The mean of the IAPF also differed significantly among the levels of CPA ($F_{2,97} = 6.658$; $p = 0.002$). As it can be observed in Fig. 1, subjects with high CPA showed the highest mean of the IAPF, while those with low CPA exhibited the lowest. Bonferroni pair-wise post-hoc tests revealed a significant difference between subjects with high and moderate levels of CPA ($p = 0.009$), and between subjects with high and low levels of CPA ($p = 0.002$). No differences emerged between moderate and low groups ($p = 0.689$). Finally, educational attainment did not exert a significant influence on the IAPF (linear regression *t*-test, $t_{98} = 1.205$; $p = 0.231$).

According to these exploratory analyses; gender, age, *APOE* genotype, and CPA exerted a significant influence on IAPF, and

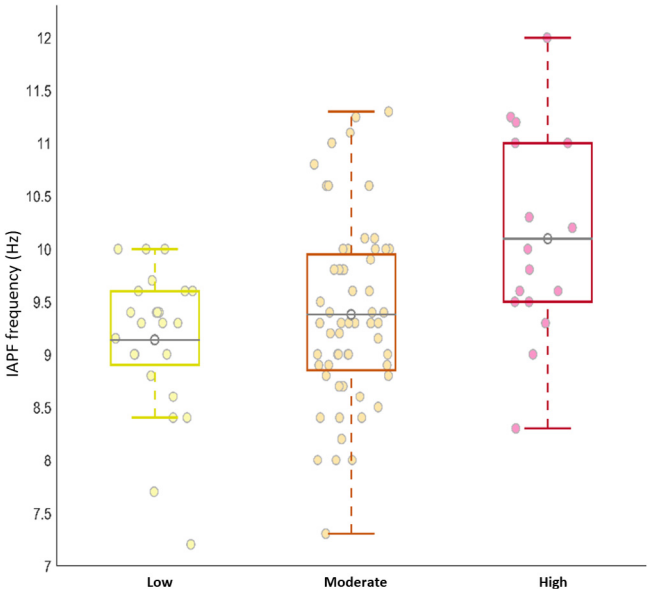

Fig. 1. The figure displays the individual values for the IAPF in each level of Categorized Physical Activity (CPA). The horizontal line inside the boxplot represents the mean IAPF for each group. The points are slightly scattered across the X-axis for visualization purposes.

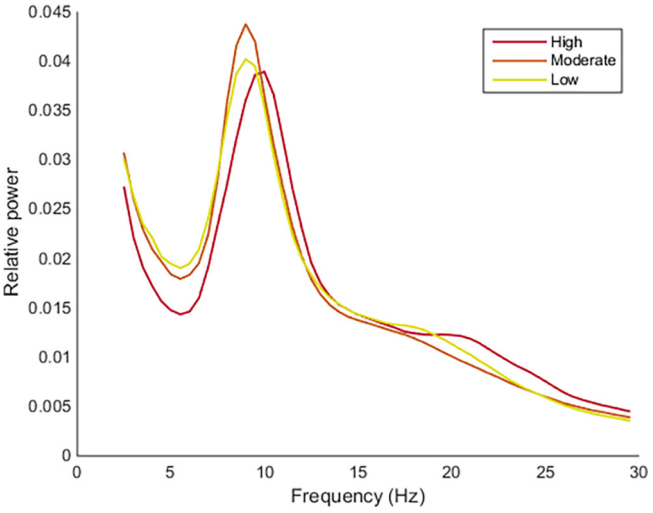

Fig. 2. The figure displays the mean power spectrum for each group of PA for an average of posterior sensors.

consequently they are candidates to be submitted to the GLM analyses (see below).

3.2. Relationship among age, gender, APOE genotype and physical activity

Age was not affected by the effect of gender (Students' *t*-test, $t_{98} = 1.625$; $p = 0.107$), *APOE* genotype (Students' *t*-test, $t_{98} = 0.535$; $p = 0.594$) or CPA ($F_{2,97} = 0.022$; $p = 0.979$). *APOE* ε4 was homogeneously distributed in both genders (Chi-square test, $\chi^2_1 = 0.012$; $p = 0.914$), and in the three levels of CPA ($\chi^2_2 = 2.448$; $p = 0.294$). Finally, gender was also homogeneously distributed in the three levels of CPA ($\chi^2_2 = 1.804$; $p = 0.406$). According to these results, it can be concluded that there exists a correct balance in the four potential prognostic variables (i.e. gender, age, *APOE* genotype, and CPA), and consequently no confounding or spurious effects should be expected.

3.3. General lineal model building

Means of the IAPF scores according to CPA levels and *APOE* genotype are displayed in Fig. 3. An intuitive interpretation of Fig. 3 information suggests that, while *APOE* ε4 non-carriers exhibit higher values of the IAPF for all levels of CPA, the differences between carriers and non-carriers in terms of medium values of the IAPF augmented as the intensity of PA increased (0.2315 Hz for low PA, 0.5778 Hz for moderate PA, and 0.8958 Hz for high PA), always favoring non-carriers. Such intuitive impressions should be confirmed by performing a two-way ANCOVA with one covariate.

Results of such test indicated that even after adjusting for age ($F_{1,93} = 4.882$; $p = 0.030$), mean IAPF was still significantly affected by *APOE* genotype ($F_{1,93} = 5.993$; $p = 0.016$) and CPA ($F_{2,93} = 4.352$; $p = 0.015$) effects. The partial eta-squared values were 0.086 for physical activity and 0.050 for *APOE* genotype, while the adjusted-R^2 was 0.179. The inclusion of gender did not result in any improvement of the model. Notably, in this model (henceforth called Model 1) the interaction between *APOE* genotype and CPA did not exert a statistically significant influence on the IAPF ($F_{2,93} = 0.531$; $p = 0.590$), but such p-value coincided with a remarkably low observed power ($p = 0.135$). At this point it is important to highlight that low observed power is associated with an increased risk of type-2 error, and consequently with an increased risk of considering as non-significant an actually significant effect. This crucial fact suggests the exploration of some alternative possibilities.

A reasonable alternative is the calculation of a one-way ANCOVA, including only two variables: age (covariate) and the *APOE* genotype x CPA interaction. Results of this new model (henceforth called Model 2) indicated that even after adjusting for age ($F_{1,93} = 4.882$; $p = 0.030$), the *APOE* genotype x CPA interaction exerted a strong effect on the IAPF values ($F_{5,93} = 4.373$; $p = 0.001$). The partial eta-squared value was 0.19 for the *APOE* genotype x CPA interaction. Of note, the *APOE* genotype x CPA interaction in Model 2 showed a remarkably higher observed power ($p = 0.958$) as compared with Model 1 ($p = 0.135$), thus supporting the improved robustness of the alternative model. The adjusted-R^2 was 0.179. According to these evidences we consider Model 2 as the final model.

Once a final model was established, we performed a series of *post-hoc* comparisons. Firstly, we analyzed differences in the IAPF scores due to *APOE* genotype within each level of the CPA variable. Results indicated that no differences emerged for the low level of PA ($p = 0.530$), while nominal differences ($p = 0.058$) appeared for the moderate, and significant differences ($p = 0.048$) appeared for the high level of CPA. Overall, *APOE* non-carriers always exhibited higher IAPF scores but the differences were more evident in the moderate, and especially in the high level of CPA. Secondly, we analyzed differences in the IAPF scores due to CPA effects separately within each *APOE* genotype group. When CPA levels were analyzed within *APOE* ε4 carriers no differences emerged (all p-values > 0.9). Importantly, when CPA levels were compared within *APOE* ε4 non-carriers a new scenario emerged. The low vs. moderate comparison was not significant ($p = 0.714$), but the low vs. high and the moderate vs. high comparisons ($p = 0.001$ and $p = 0.005$, respectively) were strongly significant (see Fig. 3). These results suggested that the effect of a higher IAPF associated with a more intense PA is only significant within the non-carriers group. Table 3 summarizes post-hoc analyses of model 2. Additionally, to further support this interaction, we conducted a Spearman correlation test between IAPF and CPA values, separately for each *APOE* group. We observed that healthy non-carriers showed a significant association between both variables (rho = 0.33; $p = 0.003$). On the contrary, no significant correlation was found within APOE ε4 carriers, and most importantly, the correlation value was notably lower (rho = 0.11; $p = 0.64$).

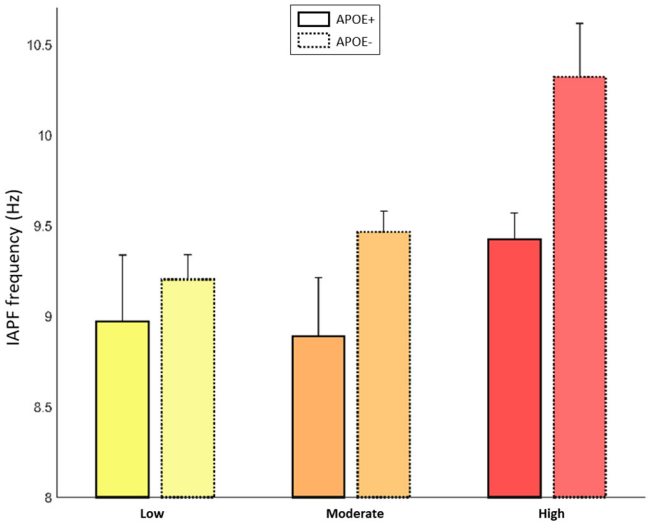

Fig. 3. The figure displays the estimated IAPF means for each subgroup of *APOE* genotype and categorized physical activity (CPA) with a black arrow indicating the standard error of the mean. For each level of CPA, the left bar (continuous line) represents the IAPF for the carriers of the APOE ε4 allele and the right bar (dotted line) represents the IAPF for the non carriers.

Table 3
This summarizes the statistical results of the final model 2, including Bonferroni post-hoc comparisons that assessed *APOE* genotype × categorized physical activity (CPA) interaction. Significant p-values are marked in bold.

Dependent variable	APOE Genotype × CPA interaction	p-value
IAPF	*APOE* genotype effect in low level of CPA	0.530
	APOE genotype in moderate level of CPA	0.058
	APOE genotype in high level of CPA	**0.048**
	CPA effect in APOE ε4 carriers	
	Low < moderate	0.977
	Low < high	0.984
	Moderate < High	0.966
	CPA effect in APOE ε4 non-carriers	
	Low < moderate	0.714
	Low < high	**0.001**
	Moderate < High	**0.005**

IAPF: Individual Alpha Peak Frequency; *APOE* ε4 = Apolipoprotein E epsilon 4; CPA: Categorized Physical Activity.

4. Discussion

The results of this study confirmed previously reported evidences and enabled the achievement of our main two goals, thus offering new insights into the combined effects of PA and *APOE* genotype. Our final statistical model concluded that the IAPF was significantly influenced by the effect of three factors: CPA, age, and *APOE* genotype. With regards to the isolated effect of CPA, our first objective was to determine whether Gutmann et al.'s (2015) findings in healthy young adults could be extrapolated to a healthy aged sample. Gutmann et al. (2015) acquired resting-state EEGs and reported a significant increase of the IAPF values in post-exercise measures within an exhaustive exercise protocol, while no differences emerged in a steady-state condition. Basically, Gutmann et al.'s experiment measured the acute effects of PA and demonstrated that the IAPF was only modified by an intense exercise. Our experiment relied on self-reported levels of physical exercise in the past seven days, and therefore we measured chronic rather than acute PA effects. However, although the experimental protocols were quite different, our findings confirmed that the IAPF values significantly increase when subjects are engaged in relatively high levels of PA (see below). In addition, our results supported previous studies where PA exerted a significant influence on brain activity within the alpha range in chronic (see for example Dustman et al., 1994; Dustman et al., 1990) and acute (see for example Crabbe and Dishman, 2004; Fumoto et al., 2010) conditions.

Age also played a significant role as covariate and showed an inverse linear relationship with the IAPF. This is quite an expectable finding. The frequency of the alpha rhythm increases from early childhood to adolescence, reaching a peak value of approximately 10 Hz by the age of 20 years, and slowly decreases during adulthood and late life (Dustman et al., 1999; Marshall et al., 2002; Niedermeyer and Lopes da Silva, 2004; Chiang et al., 2011). Garces et al.'s (2013) findings also supported the negative relationship between age and the IAPF in elder subjects. CPA and age influences were important to explain variations of the IAPF but our second, and perhaps more important goal, was to evaluate *APOE* genotype effects. Results indicated that *APOE* ε4 carriers exhibited overall lower IAPF values as compared with non-carriers. This evidence is in line with previous investigations where *APOE* ε4 carriage was associated with a slowing of the background activity. For example, Lehtovirta et al. (1996) reported that AD patients had higher theta and lower beta amplitude, with *APOE* ε4 carriers showing an "extra" slowing. Babiloni et al. (2006) found that MCI and AD patients carrying the ε4 allele showed lower alpha 1 and alpha 2 amplitudes in occipital, temporal and limbic areas. In a recent MEG study by our group, Cuesta et al. (2015) supported Lehtovirta et al.'s findings of an "extra" slowing in *APOE* ε4 carriers with cognitive deterioration. Authors performed a source analysis of spectral variations in MCI subjects and aged controls and found that MCI carriers showed the most significant slowing.

At this point it is crucial to highlight that EEG and MEG studies on *APOE* genotype share a common perspective: the spectral variations observed in carriers are mainly due to the damage that *APOE* ε4 produces on the cholinergic system. Poirier et al. (1995) and Soininen et al. (1995) demonstrated in post-mortem studies that *APOE* ε4 carriage was associated with a dose-dependent reduction of choline acetyltransferase. Chapman and Michaelson (1998) supported those evidences in animal models by reporting that *APOE*-deficient specimens showed a reduced synaptic density in the septo-hippocampal and nucleus-basalis cholinergic pathways. Notably, seminal studies such as Steriade's (2001) revealed that cholinergic projections to the cortex modulate the oscillatory neural activity. Nowadays, it is well-known that reduced cholinergic levels are associated with a significant slowing of the spontaneous background activity (Neufeld et al., 1994; Osipova et al., 2003; Riekkinen et al., 1991; Riekkinen et al., 1990).

APOE ε4's influences on cholinergic system might be of key importance to explain the meaning of the significant interaction between CPA levels and *APOE* genotype detected in our study (see below). Such interaction revealed two consistent evidences: 1. When *APOE* genotype effects on the IAPF were assessed within each level of the CPA variable, results indicated that differences between carriers and non-carriers were nominally significant in the moderate level and actually significant in the high level, with non-carriers exhibiting higher scores; and 2. When PA effects on the IAPF were analyzed within each *APOE* genotype group, results demonstrated that the comparison among CPA levels was only significant within non-carriers group, with the high level of CPA yielding significantly greater IAPF scores than moderate and low levels. These evidences suggest that, although higher levels of PA were in general associated with higher IAPF (see Fig. 3), CPA was only linked to a significant change of the alpha rhythm in *APOE* ε4 non-carriers. Moreover, such modification only appeared when subjects were habitually involved in a moderate-to-high level of physical exercise. Therefore, although the cross-sectional nature of the employed dataset might not allow the establishment of a causal relationship between higher PA and increased IAPF, previous interventional studies suggested that such relationship may actually exist (Gutmann et al., 2015). Beyond that, an alternative hypothesis could be that *APOE* status directly affects PA levels, so that the lower IAPF found in carriers were associated to lower PA levels in this group as a consequence of *APOE* ε4 carriage. Nevertheless, the fact that carriers and non-carriers did not differ in their levels of physical activity (CPA levels were homogeneously distributed between groups and mean METs were also similar) is coherent with the idea that physical activity and *APOE* ε4 carriage independently influence IAPF.

The above presented affirmations immediately give rise to two crucial questions: 1. Which underlying mechanisms may explain the observed effects of PA on brain functioning? And 2. Could these mechanisms explain why those effects seemed to be restricted to *APOE* ε4 non-carriers? With regards to the first question, Kennedy et al. (2017) accomplished an excellent review in which all the potential mechanisms that have been proposed to explain the protective effects of PA on brain function were described. Authors point out that PA has been associated with several processes such as the protection from vascular dysfunction and more particularly from arterial stiffness, the preservation of the endothelial function that ensures a stable cerebral perfusion, the promotion of neurogenesis and neural plasticity mediated by an increased expression of neurotrophines, the regulation of the hypothalamus-pituitaryadrenal system's response to stressors that may produce a chronic exposure to glucocorticoids, the prevention of exaggerated neuroinflammatory responses, and the regulation of insulin levels and the corresponding glucose uptake into neural cells.

An additional and critical process that should be added to the above detailed list is the involvement of PA in the protection and maintenance of neurons in the cholinergic system. As it has already been mentioned, PA increases the expression of neurotrophines in the brain. Interestingly, neurotrophines have also been found to promote the survival of cholinergic neurons (see Connor and Dragunow, 1998). In this line, two influential animal studies revealed the relationship between PA and the enhancement of the cholinergic system. Ben et al. (2009) demonstrated that an exercise training program of 30 days was capable to reverse the cholinergic deficit provoked by hormone deficiency in ovariectomized rats. More interestingly, Belarbi et al. (2011) investigated the beneficial repercussions of long-term PA in a transgenic mouse model of AD. Their results revealed that chronic exercise was associated with decreased hippocampal Tau pathology and with

a preservation of the expression of choline acetyltransferase within the medial septum (see also Intlekofer and Cotman, 2013). If these very relevant findings are accepted, a tentative hypothesis for the explanation of our results might be proposed. As we previously posed, the integrity of the cholinergic system is crucial for the modulation of EEG and MEG signals, and damage to this system (such as the one produced by the carriage of the *APOE* ε4 allele) causes a significant slowing of the background spectrum. Thus, it might be hypothesized that the enhancing influence of exercise on cholinergic functioning was capable to produce a weak increase of the IAPF in the group of *APOE* ε4 carriers with high levels of PA, but that increase was not sufficient to overcome *APOE* ε4's noxious effects and did not yield a statistically significant effect. On the contrary, PA was not counteracted by any detrimental influence within non-carriers, and consequently the potentiating influence of exercise was capable to produce a significant modification of the IAPF in the expected direction.

This tentative explanation of our results might be considered as quite limited, but it is important to emphasize that several research works that measured different cognitive and biological markers also reported PA effects that were restricted to or that were more pronounced in *APOE* ε4 non-carriers. Perhaps the first and more influencing study was performed by Podewils et al. (2005). Authors analysed the association between PA and risk of dementia in a sample of aged subjects, and reported that the highest levels of physical energy expenditure were correlated with reduced risk of AD only in non-carriers. Subsequent investigations revealed parallel tendencies in amyloid deposition levels (Head et al., 2012), cognitive performance (Runge et al., 2014), and risk of progression to AD (Yang et al., 2015). Notwithstanding, it is necessary to admit that even more numerous investigations reported PA effects that were more pronounced in *APOE* ε4 carriers (see for example Cerin et al., 2017; De Marco et al., 2015; Smith et al., 2016; Tolppanen et al., 2015). Here it is important to keep in mind that high levels of PA were capable to increase IAPF values even in *APOE* ε4 carriers, although such increase failed to reach a statistical significance. In fact, we cannot completely rule out the possibility that employing a larger sample of highly active carriers would yield a significant effect of PA on IAPF among APOE ε4 carriers as a consequence of increasing statistical power. Despite that, the tendency towards a lower effect of PA on IAPF in carriers compared to non-carriers seems reasonable according to our results.

At this point, is it possible to define a common denominator that might help to understand this apparent contradiction? Smith et al. (2013) claimed that the majority of studies that assessed the combined influences of PA and *APOE* genotype shared a common feature: the harmful effect of *APOE* ε4 carriage was exacerbated by physical inactivity, with less active carriers always showing the worst outcome. Such clear tendency, that is also present in our findings, might have an evolutionary significance for humans' lifespan. Raichlen and Alexander (2014) published a fine article that tried to clarify some of the reasons for the exceptionally long lifespan of humans as compared to other mammals and a potential relationship with *APOE* genotype. In particular, authors cited Fullerton et al.'s (2000) study in which it was shown that ε4 was the ancestral *APOE* allele in humans and the emergence of the ε2 and ε3 isoforms occurred between 200,000 and 300,000 years ago. For a long period, the ε4 allele was dominant in our ancestors producing its well-known pernicious effects but paradoxically humans' lifespan increased. According to Raichlen and Alexander's point of view, this tendency to more extended lifespans was due to the evolution of an increased PA among hunter-gatherers that relaxed *APOE*-related constraints on human aging. This very attractive perspective might offer a reasonable interpretation of the common finding of significantly worse outcomes in sedentary *APOE* ε4 carriers.

Finally, our study was limited by its cross-sectional rather than longitudinal design that prevented the analysis of long-term effects of PA. It would also be interesting to consider the usage of devices such as accelerometers or actigraphy to objectively measure PA instead of collecting self-reported measures. Nevertheless, several previous studies demonstrate that the IPAQ is a valid tool for measuring physical activity (Brown et al., 2004). Also, although exercise habits might not be as stable long-term as nutritional patterns, it has been proved that self-reported measures of a regular week and self-reported measures of the last seven days significantly correlate (Craig et al., 2003). Furthermore, objective measures of PA are neither exempt of biases, since they underestimate energy expenditure during biking or swimming, for example, and volunteers could become more aware of the importance of PA as a consequence of participating in these kinds of projects or simply increase their regular levels of PA while they are being monitored.

Beyond that, although our sample is composed of 100 participants, certain subsample sizes are relatively small, especially among *APOE* ε4 carriers, thus limiting an ampler generalization of the results. In such circumstances, it is not possible that to rule out the possibility that various confounding variables could mediate the observed effects. However, the factors that are generally known for having an impact on our variables of interest and therefore the ones that are usually considered in other studies have been controlled for in this study (for example educational attainment, cognitive status gender or age; See Voss et al., 2010; Killgore et al., 2013; Gutmann et al., 2015). Still, our investigation still offers the first neurophysiological evidences on the combined effects of *APOE* genotype and PA in healthy elders. Such evidences might shed some light into the modulating influences that *APOE* exerts on factors that might prevent brain deterioration. Future lines of research could also include MCI and AD patients, since this study only included a sample of elders at increased risk of AD, but with no evidence of current undergoing neurodegenerative process.

5. Conclusions

This study demonstrates that the regular practice of high levels of PA is associated with higher IAPF in healthy elders, especially within *APOE* ε4 non-carriers. The relevance of this finding lays on the fact that higher IAPF values have been found to correlate with better cognitive performance in the aged population. Therefore, our results suggest that PA-based interventions could promote cognitive health in older adults. As for the advisable amounts of exercise that could be recommended for this population, more research is needed to determine how volume and intensity of PA distinctively contribute to changes in IAPF, and consequently in brain functioning. Thus, considering the scoring criteria for the IPAQ, we may conclude that daily activity reaching a total of 3000 MET per week or at least three session of vigorous physical activity reaching a total of 1500 MET per week, should be the basic guidelines upon which planned interventions may be designed.

Disclosure statement

The authors have no conflicts of interest to disclose.

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