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ABSTRACT

Understanding the role of neuromodulators of socio-affective processing is important to ensure psychological wellbeing during older years. Here, we investigated the link between blood serotonin levels and brain and behavioral responses to emotional information in healthy elderly. A priori regions of interest (ROI) were selected due to their role in emotion processing and their dense serotonergic innervation. Correlation analyses were performed between ROI-specific responses to emotional stimuli and whole blood serotonin levels. We found significant negative associations between serotonin and functional activity for the bilateral insula, dorsal anterior cingulate cortex and subgenual gyrus. No association with behavioral measures survived correction for multiple testing. Our results mirror prior pharmacological and genetic work on the link between serotonin and emotional brain reactivity in younger adults. Given the involvement of serotonin in several age-related changes, our study encourages future research to characterize the role of this neuromodulator in emotion processing across the lifespan.

1. Introduction

The past few years have witnessed a growing number of biomedical interventions aimed to increase human life expectancy. These efforts, however, are focused on the quantity of living years, while the quality is still under threat of mood disorders and dementia (Brown, 2015). Along with the accumulation of molecular, cellular and organ damage, ageing is characterized by a loss of brain weight and a decline in neuronal volume (Peters, 2006). This has a direct impact on neurotransmitter concentrations (Amenta, Zaccheo, & Collier, 1991) and may underlie the behavioral and cognitive changes commonly observed in the elderly population (Peters, 2006). Due to their involvement in neuro-degeneration, modifications in dopaminergic and catecholaminergic circuits are the focus of abundant ageing research (Amenta et al., 1991; Carlsson, 1987), whereas other monoamine systems, such as serotonin, are less investigated. Nonetheless, serotonin is considered as one of the main endogenous modulators of socio-affective states and emotion (Canli & Lesch, 2007; Kiser, Steemers, Branchi, & Homberg, 2012), acting through widespread projections to cortical and limbic regions (Hornung, 2003). Given the importance of emotional wellbeing for a healthy ageing process (Steptoe, Deaton, & Stone, 2015), the role of this neurotransmitter in socio-affective processing during late life deserves special attention.

More than 90% of the total serotonin (5-hydroxytryptamine [5-HT]) in the human body is synthesized in the gut from where it is distributed by circulating platelets to various body sites (Berger, Gray, & Roth, 2009). The remaining amount of body 5-HT is found in the central nervous system, where it is synthesized in the midbrain raphe nuclei (Hornung, 2003). Prior studies offer contrasting evidence regarding changes in 5-HT availability, synthesis, and transmission during normal ageing. For instance, some studies have described decreases in 5-HT receptors and transporters in the human brain, which results in an overall reduced serotonergic neurotransmission in healthy ageing (for a review, see Karrer, McLaughlin, Guaglianone, and Samanez-Larkin, 2019). On the other hand, other human and animal investigations observed that 5-HT levels remain unaltered during healthy ageing (Goldman-Rakic & Brown, 1981; Rosa-Neto et al., 2007), suggesting that despite reductions in specific 5-HT subsystems (e.g., 5-HT receptors),

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serotonergic neurotransmission is not affected across the lifespan (Morgan, May, & Finch, 1987). It is generally accepted, however, that lower brain 5-HT availability is associated with geriatric depression (Nobler, Mann, & Sackeim, 1999) and may exacerbate behavioral symptoms in Alzheimer disease (Lanctot, Herrmann, & Mazzotta, 2001; Meltzer et al., 1998; Morgan et al., 1987). Similarly, decreases in peripheral measures of 5-HT such as blood 5-HT (i.e., whole blood, plasma, and platelets) have been described in mood and other neuropsychiatric disorders in older adults (Gao, Zhu, Zhang, & Wang, 2008; Kumar, Sevush, Kumar, Ruiz, & Eisdorfer, 1995). Even if the trajectory of 5-HT function across the lifespan is still to be clarified, converging data suggest an implication of 5-HT in a wide range of age-related changes, including sleep patterns (Myers & Badia, 1995), mood (Alexopoulos, 2019), and cognition (Myers & Badia, 1995; Peters, 2006) among others. In the current study, we specifically aimed at investigating whether individual differences in whole blood 5-HT levels are associated with changes in emotion-related behavioral and neural measures in a healthy elderly sample.

Even though circulating 5-HT cannot cross the blood-brain barrier and may not reflect true brain serotonergic function, prior human and animal research described a positive relationship between 5-HT transporter levels in the human brain and in blood platelets (Rausch et al., 2005), as well as simultaneous increases in both blood and brain 5-HT levels of rats after the administration of tryptophan (i.e., 5-HT precursor; Collins, Kloek, and Elliott, 2013)). Similarly, other studies reported very high correlations (i.e., Pearson's r = .57–.97) between blood 5-HT levels and 5-HT in cerebrospinal fluid (CSF) in both animals (Audhya, Adams, & Johansen, 2012) and humans (Audhya et al., 2012; Gao et al., 2008). Given that 5-HT levels in CSF may accurately reflect serotonergic activity in the central nervous system (Matsumoto et al., 1991), these findings indicate that peripheral measures of 5-HT may serve as a valid proxy for brain serotonergic availability. Specifically, whole blood 5-HT is considered as a good estimate of platelet serotonin, which contains 99% of the total serotonin in whole blood (Cleare, 1997). Functionally, human studies have observed that higher blood 5-HT levels are associated with higher mood scores in healthy young individuals (Williams et al., 2006) and predict positive responses to serotonergic antidepressants (Holck et al., 2019). On the other hand, lower levels of whole blood 5-HT have been reported in mood disorders and other psychiatric conditions (Banki, 1978; Cleare, 1997; Mann, Mcbride, Anderson, & Mieczkowski, 1992), evidencing a certain vulnerability of the 5-HT system. Particularly relevant for the current study, peripheral measures of 5-HT in blood tend to decrease with age (Jernej et al., 2000; Taborskaya, Frolova, & Kuleva, 2016). Yet, it remains unclear whether blood 5-HT levels in the elderly display the same association with emotional measures as observed in younger cohorts.

A large body of pharmacological functional magnetic resonance imaging studies (pharmaco-fMRI) have described how different levels of 5-HT can modify functional brain responses to emotional stimuli. For example, Kramer and colleagues reported that lower 5-HT levels (induced by acute tryptophan depletion) were associated with diminished aggression and less insular activation during a competitive reaction time task (Kramer, Riba, Richter, & Munte, 2011). Similarly, recreational ecstasy users (as a model of 5-HT depletion) were studied with PET and fMRI during a face processing task, revealing a dose-dependent activation in the amygdala during the processing of emotional faces (Laursen et al., 2016). Likewise, the fMRI meta-analysis of Outhred and colleagues showed that single dose of 5-HT reuptake inhibitors (SSRI) can decrease amygdala activity and modulate frontal activity during the presentation of emotional stimuli (Outhred et al., 2013). Besides serotonergic challenges, genetic variations may also regulate 5-HT neurotransmission and availability (i.e., 5-HTTLPR (Kobiella et al., 2011); MAO-A (Eisner et al., 2017)) with corresponding differences in fMRI responses in corticolimbic regions (i.e., amygdala, insula and dorsal cingulate cortex) during the regulation of emotional arousal. Interestingly, these and other fMRI studies found differential activation in brain structures that are not only involved in emotion processing, but also known to exhibit rich 5-HT innervation (Deza-Araujo et al., 2019; Klumpp et al., 2014; Robinson et al., 2013). This suggests that 5-HT availability could play an important role in regulating the function of these brain structures. Furthermore, different levels of circulating 5-HT measures are also described in association with changes in brain activity or connectivity, further supporting the notion of a serotonergic regulation (Deza-Araujo et al., 2019; Eisner et al., 2017). While all the work mentioned above was carried out in young adults, no study has dealt with similar association in healthy older cohorts.

Here, we examined a set of a priori defined brain regions with functional magnetic resonance imaging in order to investigate the relationship between whole blood 5-HT and brain responses to affective stimuli in older adults. Regions of interest (ROIs) were selected based on their dense serotonergic innervation and their involvement in emotional and socio-affective reactivity: amygdalae, insulae, dorsal anterior cingulate cortex (dACC), and subgenual cingulate gyrus (sgCG). Brain responses to affective stimuli were elicited using the Socio-affective Video Task-Rest (Baez-Lugo et al., 2021; Klimecki, Leiberg, Lamm, & Singer, 2013). Based on previous fMRI work summarized above, we hypothesized a negative correlation between 5-HT levels and neural responses in the aforementioned ROIs to highly emotional stimuli. On a behavioral level, we expected 5-HT levels to be negatively correlated with measures of anxiety, depression, worry, rumination, and negative affect ratings elicited by the stimuli. Given contrasting reports on the involvement of 5-HT in empathy and prosocial behaviors (Canli & Lesch, 2007; Kiser et al., 2012), we explored the relationship between whole blood 5-HT and behavioral measures of these social variables. Lastly, we explored the association between the functional responses of selected ROIs and all behavioral measures of emotion and prosocial behaviors.

2. Experimental procedures

2.1. Participants

This dataset was part of the baseline assessment of the Age-Well randomized clinical trial (Poisnel et al., 2018), a part of the Medit-Ageing study (https://silversantestudy.eu). Participants were recruited from the community of Caen (Normandy, France) via advertisement. Individuals who expressed interest in participating were invited to a screening session. The detailed recruitment process, as well as the inclusion and exclusion criteria are described in Supplemental Material S1 and S2 respectively. A total of 135 cognitively unimpaired healthy elderly were included in the baseline assessment, which comprised two study visits (an MRI scan and a PET scan). We discarded five participants due to scanner motion (see Methods section), three due to scanner artifacts, one due to excessive Body Mass Index (BMI > 43), and seven participants who declared self-medication against migraine, low mood or insomnia (which might interfere with serotonergic function) more than once a week. Thus, our final sample comprised data from 119 participants (72 females; M_{age} = 68.9 years, SD_{age} ± 3.73, Min_{age} = 64, Max_{age} = 83). Sensitivity analyses showed that our sample size provided sufficient power ($1-\beta = 0.80$) to detect small-sized effects ($dz = 0.22$) at $\alpha = 0.05$ (Faul, Erdfelder, Lang, & Buchner, 2007).

All participants provided informed consent and received monetary compensation. The Age-Well randomized clinical trial, sponsored by Inserm, was approved by the ethics committee (Comité de Protection des Personnes Nord-Ouest III, Caen, France; trial registration number: EudraCT: 2016−002441-36; IDRCB: 2016-A01767−44; ClinicalTrials.gov Identifier: NCT02977819).

2.2. Whole blood serotonin extraction and analysis

Fasting sampling was performed in the morning after one day of diet excluding tryptophan-rich food (e.g., dried fruits, avocados, nuts,

chocolate, bananas). Blood samples were collected into heparinated-containing Vacutainer tubes from participant by intravenous cannulation. The samples (2 mL of total blood) were immediately frozen at −20 °C within the hour from collection.

The concentrations of 5-HT in whole blood were determined using high-performance liquid chromatography method with colorimetric detection. The analyses were separated in 10 min on a reversed-phase column (C18) with phosphate buffer (pH 5. 5)-methanol (90:10, v/v) as mobile phase. The flow rate was 1.3 mL/min. The colorimetric measurements were carried out at 50 mV for the first detector and at 300 mV for the second one, which allowed the determination of 5-HT levels. Whole blood 5-HT values were expressed in μmol/L and standardized into Z-scores for statistical analyses. For comparison with other studies, μmol/L values of whole blood 5-HT were converted to ng/mL.

2.3. Behavioral assessment

All participants completed a comprehensive assessment with neuropsychological, socio-affective, and biological measures (see Poisnel et al. (2018) for a complete description). The behavioral variables reported in this study focused on the assessment of emotional and psycho-affective states, as well as empathy and prosocial behavior due to their connection with the task and 5-HT. Questionnaires included: the Penn State Worry Questionnaire (PSWQ. Possible range of scores: 16–80; (Meyer, Miller, Metzger, & Borkovec, 1990), French version (Gosselin, Dugas, Ladouceur, & Freeston, 2001)); the Rumination Response Scale (RRS. Possible range of scores: 22–88; (Treynor, Gonzalez, & Nolen-Hoeksema, 2003), French version (Parola et al., 2017)); the State-Trait anxiety Inventory (STAI. Possible range of scores: 20–80; (Spielberger, 1984), French version (Gauthier & Bouchard, 1993)); the Geriatric Depression Scale (GDS. Possible range of scores: 0–30; (Yesavage et al., 1983), French version (Clement, Nassif, Leger, & Marchan, 1997)); the Interpersonal Reactivity Index (IRI – Distress, IRI- Empathic Concern, IRI- Perspective Taking, IRI- Fantasy. Possible range of scores in each subscale: 4–49; (Davis, 1983), French version adapted from (Gilet, Mella, Studer, Gruhn, & Labouvie-Vief, 2013)); and the Prosocialness scale (Possible range of scores: 16–80; (Caprara, Steca, Zelli, & Capanna, 2005), French version (Carrizales, Perchec, & Lannegrand-Willems, 2019)).

2.4. Functional task and stimuli

To assess brain activity in response to different categories of socially and emotionally significant stimuli, we used a modified version of the Socio-affective Video Task (SoVT; (Klimecki et al., 2013)). Briefly, this task includes blocks of three sound-free short videos (10–18 seconds) depicting people in suffering situations (high emotion; HE) and people in neutral everyday situations (low emotion; LE). The task comprises 12 HE videos and 12 LE videos grouped in interleaved blocks of three HE and three LE videos, divided into two runs. In addition, resting periods of 90 s were included after each block of both HE and LE videos to assess the degree to which emotions persisted in time (SoVT-Rest. Fig. 1) after emotion elicitation (Eryilmaz, Van De Ville, Schwartz, & Vuilleumier, 2011). A detailed description of the SoVT-Rest is presented in Supplemental Material S3. Main results of the full fMRI protocol and its validation in our elderly population are reported elsewhere ((Baez-Lugo et al., 2021)).

After the fMRI session, participants watched the videos again outside the scanner and rated subjective levels of empathy ("To what degree did you feel the emotions of the characters"?), positive emotions ("Indicate

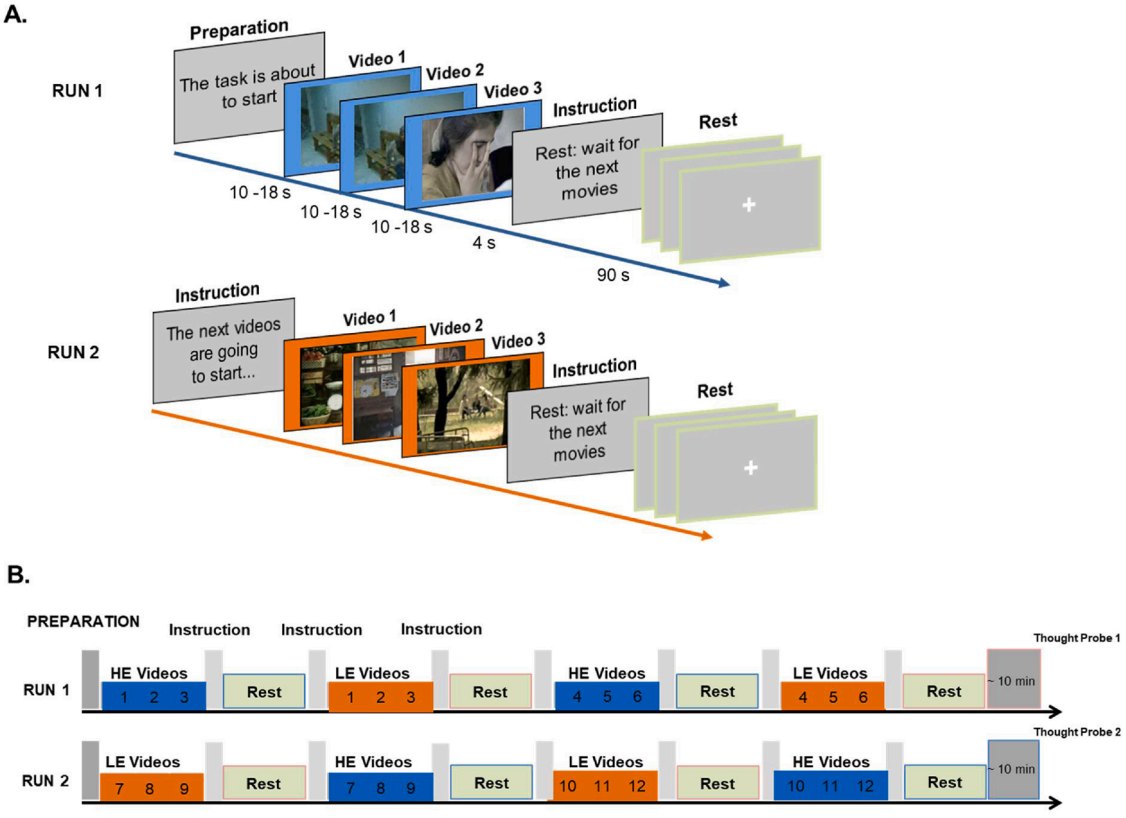

Fig. 1. SoVT-Rest paradigm adapted from Baez-Lugo et al. (under review). **A.** The task presented a block of three short sound-free videos, depicting either people in distress (i.e., High Emotion – HE) or people in everyday situation (i.e. Low Emotion – LE). A resting-state period of 90 s followed each block of videos. **B.** The task performed in the scanner comprised two runs, randomized across participants. The lower part of the figure depicts the structure of both runs. At the end of each run, a thought probe was performed (data reported in (Baez-Lugo et al., 2021)) to assess differences in spontaneous thought content during the last resting period (one after HE videos and one after LE videos).

the intensity of your positive emotions") and negative emotions ("Indicate the intensity of your negative emotions") using a scale with anchors at 0 = "not at all" and 10 = "extremely". To compare emotions in response to either the HE or the LE videos, a one-way analysis of variance (ANOVA) was conducted with "video" (i.e., HE and LE) as a within subject factor and "emotion" (i.e., empathy, positive affect, negative affect) as a dependent variable. Since sphericity assumptions were not met, Greenhouse-Geisser correction was used for reporting the results. If the F-test indicated significant differences, post-hoc paired *t*-test were used to determine the directionality of the effects. Significance was considered at $p < .05$, after correction for multiple comparisons using False Discovery Rate (FDR).

2.5. MRI acquisition and preprocessing

Structural and functional scanning was performed on a Philips (Eindhoven, The Netherlands) Achieva 3.0 T scanner from the GIP Cyceron (Caen, France). BOLD fMRI during the SoVT-Rest was acquired in two runs, with a T2*-weighted asymmetric spin-echo echo-planar sequence (~ 22 min; TR = 2.000 ms, TE =30 ms, flip angle = 85°, FOV = 240 × 240 mm2, voxel size = 3 × 3 × 3 mm) in the axial plane parallel to the anterior–posterior commissure, with a 32-channel head coil. Volumes were acquired, tilted slightly from axially to coronal to be parallel to the anterior/posterior commis sural line. For registration purposes, a T1-weighted high-resolution anatomical scan was acquired with a 3D fast field echo sequence (3D-T1-FFE, TR =7.1 ms, TE =3.3 ms, flip angle = 6°, 180 slices, isotropic 1 × 1 × 1 mm voxels; FoV = 256 × 256 mm^2, ~5 min) in the sagittal plane. Additionally, to correct for geometrical distortions, we collected a high resolution T2-weighted spin echo anatomical acquisition (3D-T2-SE sagittal, SENSE factor = 2; TR = 2500 ms; TE = 236 ms; flip angle = 90°; 180 slices; slice thickness = 1 mm; no gap; FoV = 250 × 250 mm^2; in-plane resolution = 0.98 × 0.98 mm^2), and a non-Echo-Planar Imaging (EPI) T2* volume (2D-T2*-FFE axial, SENSE factor = 2; TR =600 ms; TE =16 ms; flip angle = 20°; 70 slices with no gap; slice thickness = 2 mm; FoV = 256 × 256 mm^2; in-plane resolution = 2 × 2 mm^2). Participants were provided with earplugs for noise protection and foam pads to minimize head movements. The SoVT-Rest was presented on a magnetic resonance compatible screen and viewed through a rear-view mirror system. The computer package Cogent, for Matlab (http://www.vislab.ucl.ac.uk/cogent.php) was used to present the stimuli and to collect behavioral responses.

2.6. Image preprocessing and region-of-interest (ROI) definition

Before any preprocessing step, functional and structural images were visually inspected to discard susceptibility artifacts and anatomical abnormalities. Functional and structural images were preprocessed using SPM12 (Wellcome Trust Centre for Neuroimaging, London, UK). We used the procedure described by Villain et al. (2010) to improve neuroanatomical accuracy of fMRI results localization. Briefly, functional data was realigned to the first volume. Next, the T1, T2, and non-EPI T2* were co-registered to the mean EPI volume. After this, the mean EPI image was warped to roughly match the non-EPI T2* volume. All deformation parameters obtained from this co-registration were applied to the EPI volumes. T1 images were segmented using the MNI priors and the resulting parameters were applied to the co-registered T1, EPI, and non-EPI T2* volumes for normalization into MNI space. Lastly, the resulting normalised EPI images were spatially smoothed with an isotropic Gaussian kernel (full width at half maximum = 8 mm).

To account for the effects of motion on the functional data, framewise displacement (FD) measures were calculated (Power, Barnes, Snyder, Schlaggar, & Petersen, 2012). Five participants with more than 10% of volumes over a FD of 0.5 mm were excluded from the subsequent analyses. Additionally, volumes with a FD > .5 mm were flagged to be censored during the first-level statistics. This procedure censored, on average, 6.47 volumes in the 62 participants that were affected in the total sample, the remaining 57 participants had no censored volumes (total $N = 119$). As suggested by (Siegel et al., 2014) the removal of single volumes (in contrast to the removal of volumes before and after) and a threshold of FD 0.5 mm is sufficient to improve data quality in cases of moderate amounts of head movement.

First-level statistics included high-pass filtering at 256 s to account for the temporal autocorrelations (AR(1)) and low frequency drifts, and a general linear model (GLM) consisting of boxcar time series convolved with the haemodynamic response function (HRF). Specifically, the linear model included regressors for each block of three videos (~45 s) and their subsequent resting periods (90 s, divided in 3 bins of 30 s) to estimate the beta coefficients representing the corresponding brain activity in each run. Besides regressors of interest, the individual design matrices included six movement regressors, and flagged volumes with a FD > .5 mm for motion censoring.

In order to obtain the subject-specific functional brain responses to HE and LE videos and rest, we conducted the following computations: using the ImCalc function of SPM, we summed all the first-level contrasts images representing the functional brain responses to each block of HE and LE videos, as well as their corresponding resting periods (i.e., rest after HE videos and rest after LE videos). Next, the resulting summed images corresponding to the LE videos were subtracted from the summed images corresponding to HE videos, thereby evidencing the magnitude of the brain responses towards HE stimuli compared to LE stimuli. The same procedure was carried out with images corresponding to the resting periods (i.e. HE-LE videos: [i1 + i2 + i3] - [i7 + i8 + i9]. HE-LE rest: [i4 + i5 + i6] – [i10 +i11 +i12]). The resulting subject-specific images were used in the subsequent analyses.

We focused on a priori region of interest (ROI) to test for correlations between whole blood 5-HT and magnitude of functional brain responses to HE stimuli. Four ROIs were defined due to both their reported involvement in socio-affective processes and their dense serotonergic innervation, namely the bilateral amygdala (Kobiella et al., 2011; Oathes, Hilt, & Nitschke, 2015), the bilateral insula (Santangelo et al., 2019; Simmons, Arce, Lovero, Stein, & Paulus, 2009), the dorsal anterior cingulate cortex (dACC; (Faria et al., 2014; Robinson et al., 2013)), and the subgenual cingulate gyrus (sgCG; (Drevets, Savitz, & Trimble, 2008; Varnas, Halldin, & Hall, 2004). All ROIs were constructed by placing spheres on centroids of activation of these ROIs, identified in prior studies and documented in the publicly database Neurosynth (www.neurosynth.org (Yarkoni, Poldrack, Nichols, Van Essen, & Wager, 2011). To identify the coordinates of these ROIs, we used the name of each region as search terms in Neurosynth (e.g., "amygdala") and retained the MNI peaks corresponding to the most common activation site across studies. Both right and left ROIs were used for amygdala and insula, whereas a single bilateral ROI (pooling both sides across the midline) was used for dACC and sgGC (Fig. 2). Table 1 displays the center coordinates identified in Neurosynth and used for each ROI, with the diameter of each sphere in millimeters (mm)

Additional procedures were used to verify the reliability of our results. First, we repeated the above-mentioned procedures using binary anatomical masks of each region of interest created with FSLeyes (https://git.fmrib.ox.ac.uk/fsl/fsleyes/fsleyes/). Briefly, we created the same ROIs based on the anatomical locations from the Harvard-Oxford atlas (Desikan et al., 2006) for the amygdalae and insulae, and from the Brainetomme Atlas (Fan et al., 2016) for the dACC and sgCG. (See Supplemental Material S4). Second, we ran a separate whole-brain analysis where we entered whole blood 5-HT measures as covariates in a group-level analyses to see how they modulated the main effect of emotional stimuli (HE—LE contrasts) for videos and rest. (See Fig. 4).

2.7. Statistical analyses

The Marsbar software http://marsbar.sourceforge.net was used to extract mean functional activations from the abovementioned ROIs from each participant. To test our hypotheses, Pearson's correlation analyses

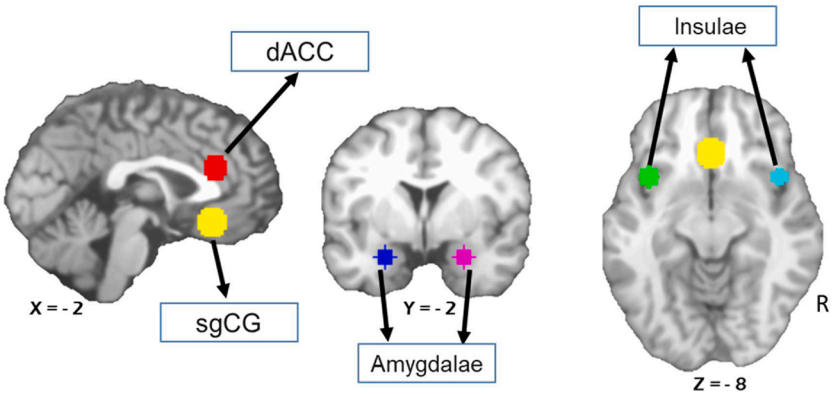

Fig. 2. Regions of interest (ROIs) selected for the analyses, namely: dorsal anterior cingulate cortex (dACC), subgenual cingulate gyrus (sgCG), bilateral amygdala and bilateral insula. Brain slices are presented in neurological orientation.

Table 1
ROI information from automated meta-analyses as specified in Neurosynth.

ROI	MNI coordinates (X, Y, Z)	Sphere size (mm)	Z-score reported in Neurosynth
Right amygdala	24, -2, -20	6	33.11
Left amygdala	−24, -4, -20	6	33.11
Right insula	40, 10, 0	6	14.05
Left insula	−38, 10, -8	6	14.20
dACC	2, 26, 22	8	12.12
sgCG	2, 26, -10	8	17.83

Search terms used to define ROIs were the name of each brain region. dACC = dorsal anterior cingulate was identified with the search term "anterior cingulate". The sphere for ROI construction was placed in the dorsal part of the region display in Neurosynth. sgCG = subgenual cingulate gyrus. Both, the dACC and the sgCG spheres were placed across the brain midline. The size of the brain region was considered when choosing the sizes of the spheres.

(zero-order and partial, adjusted for age and sex) were conducted between standardized measures of whole blood 5-HT and the resulting HE-LE activations of each ROI during videos and resting periods. The same procedure was followed for the analysis based on anatomical masks (see Supplemental Material S4).

To avoid type I errors in our planned analyses, false-discovery rate (FDR) was used to correct for multiple comparisons under the assumption of directionality and positive dependency between our variables (i.e., four ROIs in videos and four ROIs in rest) (Benjamini & Hochberg, 1995)(Benjamini and Hochberg, 1995). The same correction was applied to zero-order and partial correlations between the standardized measures of whole blood 5-HT and behavioral measures of worry, rumination, anxiety, depression, and positive and negative affect.

Exploratory analyses were also performed to relate 5-HT levels with behavioral measures of empathy and prosocial behaviors (four IRI subtests, prosocial scale and ratings of empathy from the SoVT- Rest videos). For completeness, we reported uncorrected values to describe these relationships but none survived correction for multiple comparisons. Similarly, we also reported uncorrected values for exploratory correlations between ROI activity and behavioral measures.

All analyses were conducted using R (version 3.6.1) primarily using the ppcor package (Kim, 2015).

2.8. Data and code availability

Anonymized demographic and behavioral information, brain activation parameters extracted from the ROIs and the code used to produce the main findings and figures of this manuscript are publicly available in https://data.mendeley.com/datasets/23h2jms3bs/2. Additional data are available from the Medit-Ageing group upon reasonable request and pending approval by the Consortium (https://silversantestudy.eu/2020/09/25/data-sharing/).

3. Results

3.1. Whole blood 5-HT in our sample

The values of 5-HT found in our sample (mean ± S.D. = 114.54 ± 51.10 ng/mL; min-max = 28.19–283.71 ng/mL) were within the range of whole blood 5-HT reported in a previous study with healthy older adults (Wulsin et al., 2009) although the mean of our sample was slightly lower (Our sample: 114.54 ng/mL; Wulsin and colleagues: 119 ng/mL). Values were yet lower than those reported in prior studies for healthy younger populations (N = 356 males, with mean age 21 year-old: 185.75 ng/mL, no S.D reported (Moffitt et al., 1998)); N = 36 females and 42 males with mean age 38.7 year-old: 185 ± 68 ng/ml (Leventhal, Cook, Morford, Ravitz, & Freedman, 1990)). Independent Samples t-test showed that the whole blood 5-HT values from our study were statistically different from those observed in the last study ($t(195) = -7.81$; $p < 0.01$ two-tailed).

Spearman's rank correlations showed no significant association between whole blood 5-HT and age (ρ = -0.09;p = .29). Similarly, independent t-tests showed no significant differences in 5-HT levels between males and females (p = 0.23). Nevertheless, due to previously reported differences in serotonergic availability between males and females (Jovanovic et al., 2008; Nishizawa et al., 1997), we chose to control for the variance explained by these variables in our data.

3.2. Behavioral results and participant's demographics

Participants' characteristics and descriptive statistics for all behavioral measures are reported in Table 2. The behavioral assessment did not show any extreme score in any of the questionnaires reported in this study, suggesting absence of depressive or anxious symptomatology. As some variables were not normally distributed according to Komogorov-Smirnov's test ($p < .001$), Spearman's rank correlations were used for all the analyses between 5-HT levels and behavioral measures.

Concerning the hypothesized negative association between whole blood 5-HT and measures of negative affect (worry, rumination, anxiety, depression, and negative ratings of videos) elicited by the HE condition, Spearman's correlations revealed no significant association between these variables (all $ps > .18$, uncorrected). The relationship between whole blood 5-HT and positive ratings of the LE videos (which we assumed to be mildly positive) also yielded non-significant results (p = .77, uncorrected).

Similarly, our exploratory analyses between 5-HT levels and measures of empathy and prosocial behavior did not yield any significant

Table 2
Demographic information and descriptive statistics of the sample.

		$N = 119$
Demographics	Sex	♂ = 47, ♀ = 72
	Age (years)	69.03 (3.72)
	Current smokers	$n = 7$
	Education (years)	13.24 (3.14)
	Years of retirement	8.64 (5.48)
	BMI (kg/m^2)	26.04 (4.19)
Behavioral measures	PSWQ	41.33 (11.43)
	RRS	35.11 (9.21)
	STAI-trait	34.35 (7.04)
	GDS	1.19 (1.64)
	Prosocialness scale	60.47 (8.30)
	IRI- Distress	10.23 (5.26)
	IRI-Empathic Concern	19.72 (4.18)
	IRI- Perspective Taking	17.71 (3.43)
	IRI- Fantasy	14.18 (4.57)
Video-related ratings	Positive emotions - HE	2.21 (1.52)
	Negative emotions - HE	7.34 (1.74)
	Empathy- HE	7.79 (1.50)
	Positive emotions- LE	5.61 (1.44)
	Negative emotions - LE	2.86 (1.31)
	Empathy - LE	5.71 (1.59)

Apart from sex and current smokers, all values are mean (± S.D). BMI = Body mass index; PSWQ = Penn State Worry Questionnaire; RRS = Rumination Response Scale; STAI-trait = State-Trait Anxiety Inventory; GDS = Geriatric Depression Scale; IRI = Interpersonal Reactivity Index; HE= High emotion; LE = low emotion.

results (all *p*s > .15, uncorrected). All these results are presented in the Supplemental Tables S1 and S2.

On the other hand, we found significant differences in the ratings of empathy, positive emotions and negative emotions for the HE and the LE videos ($F(5590) = 324.73$, $p < .0001$, $\eta^2 = 0.65$). Post-hoc paired comparisons showed that HE videos elicited higher empathy ($p < .001$), more negative emotions ($p < .001$), and less positive emotions ($p < .001$) than LE videos. These results validate the effectiveness of our emotion elicitation procedure. Complete results are presented in (Baez-Lugo et al., under review).

BMI = Body mass index; PSWQ = Penn State Worry Questionnaire; RRS = Rumination Response Scale; STAI-trait = State-Trait Anxiety Inventory; GDS = Geriatric Depression Scale; IRI = Interpersonal Reactivity Index; HE= High emotion; LE = low emotion.

3.3. Whole blood 5-HT and functional brain reactivity in target ROIs

Normality tests and inspection of the distributions revealed approximate normal distribution for all ROI values. Zero-order Pearson's correlations were performed between whole blood 5-HT levels and the functional brain response to HE videos (with respect to LE videos) and their corresponding resting periods. These analyses showed significant negative associations between 5-HT levels and the brain responses during HE-LE videos for the bilateral insula (right insula: $r = -0.267$,$p = .004$; left insula: $r = -0.251$,$p = .006$), the dACC ($r = -0.195$,$p = .033$) and the sgCG ($r = -0.195$,$p = .034$). Pearson's partial correlation (adjusted for age and sex) revealed similar associations between these whole blood 5-HT and the activity of these ROIs during HE videos compared to LE videos (right insula: $r = -0.266$,$p = .003$; left insula: $r = -0.249$;$p = .006$; dACC: $r = -0.207$;$p = .024$, and sgCG: $r = -0.206$;$p = .025$). All these associations remained significant after FDR correction. No significant correlation was found in bilateral amygdala. Significant FDR-corrected p-values resulting from zero-order and partial correlations are depicted in Figs. 3 A and 3B, respectively.

Pertaining to the resting periods (HE-LE), zero-order and partial correlation analyses between the whole blood 5-HT levels and brain activity in the same ROIs revealed a positive association for the left amygdala ($r = .201$, $p = .028$), albeit non-significant after FDR correction for multiple comparisons. No effect was found for the other ROIs. All FDR-corrected results are presented in Table 3.

A repetition of partial correlation analyses using the atlas-based anatomical ROIs yielded similar results (For HE-LE videos: right insula, $r = -0.231$,$p = .011$; left insula, $r = -0.285$,$p = .002$; dACC, $r = -0.192$,$p = .034$; sgCG, $r = -0.222$,$p = .014$, bilateral amygdala = *n.s.* For rest after HE-LE videos, left amygdala, $r = .202$, $p = .023$; all the other regions, *n.s.* Complete results are presented in Supplemental Material S4 and Supplemental Figure S2). However, given the larger size of anatomical regions in these atlases, which sometimes included neighbouring areas, we decided to favour the Neurosynth based ROIs. Finally, whole-brain analyses confirmed the specificity of our ROI-based results. Specifically, 5-HT measures showed a negative modulation slope of neural responses for the HE-LE contrast during videos, most prominent in the insular cortex, sgCG, and the dACC (see Fig. 4).

3.4. Behavioral variables and functional brain reactivity in target ROIs

Spearman's correlations between self-reported emotions and activations in ROIs in response to HE-LE videos revealed a positive association between activity of bilateral amygdala and questionnaire scores for rumination (RRS; Right amygdala: $\rho = .201$, p = .023; left amygdala: $\rho = 0.223$,$p = .011$) and anxiety (STAI-trait; Right amygdala: $\rho = .201$, p = .023; left amygdala: $\rho = 0.201$,$p = .023$). During the rest periods after HE-LE videos, Spearman's correlations showed a positive association between right amygdala and insula activations and empathic concern ($\rho = .182$, $p = .041$ for right amygdala and $\rho = .231$, $p = .011$ for left insula). Similarly, there were positive associations between the HE-LE activity of the right insula and IRI-fantasy ($\rho = .210$, $p = .019$) and between the left insula and IRI-distress ($\rho = .181$, $p = .041$). There were no significant association between activation in ROIs in response to HE-LE videos or rest and the difference (HE-LE) in ratings of positive or negative affect or empathy. Although these results support the validity of the dataset, these correlations are exploratory in nature, do not survive correction for multiple comparisons and should thus be interpreted as non-confirmatory. Complete results of these correlations are presented in Supplemental Tables S3 and S4.

4. Discussion

The main goal of this study was to examine the relationship between whole blood 5-HT levels and functional brain responses of emotion-related brain regions in a relatively large cohort of healthy elderly. In partial agreement with our hypotheses, lower levels of whole blood 5-HT were associated with higher activity to high versus low emotional videos in the bilateral insular cortex, dACC and sgCG. These associations were not present in the subsequent resting periods, indicating a modulation of stimulus-driven processing. In contrast, 5-HT levels did not correlate with measures of negative/positive affect or self-reports of prosocial behaviors in our sample. Lastly, exploratory analyses showed modest correlations between emotional responses (HE-LE contrast) in these ROIs and behavioral measures of socio-affective functions. Given the potential alterations of the serotonergic system with age (Karrer et al., 2019; Meltzer et al., 1998), our findings provide strong bases for future investigations on the link between 5-HT and emotion processing in elderly populations.

A crucial point in this study is the use of whole blood 5-HT levels as possible indicator of serotonergic central availability. Conventionally, 5-HT levels in blood platelets are taken as the best indicator to indirectly model serotonergic availability in the nervous system (Audhya et al., 2012). However, drastic reductions in the function of the 5-HT transporter SERT with age lead to a diminished availability of 5-HT in blood platelets in older subjects (Karrer et al., 2019; Taborskaya et al., 2016). This might prevent the use of blood platelets as a reliable serotonergic neuron model in the elderly and instead advocate the use of whole blood measures, which contains almost the total amount of 5-HT found in blood platelets (Banki, 1978; Cleare, 1997).

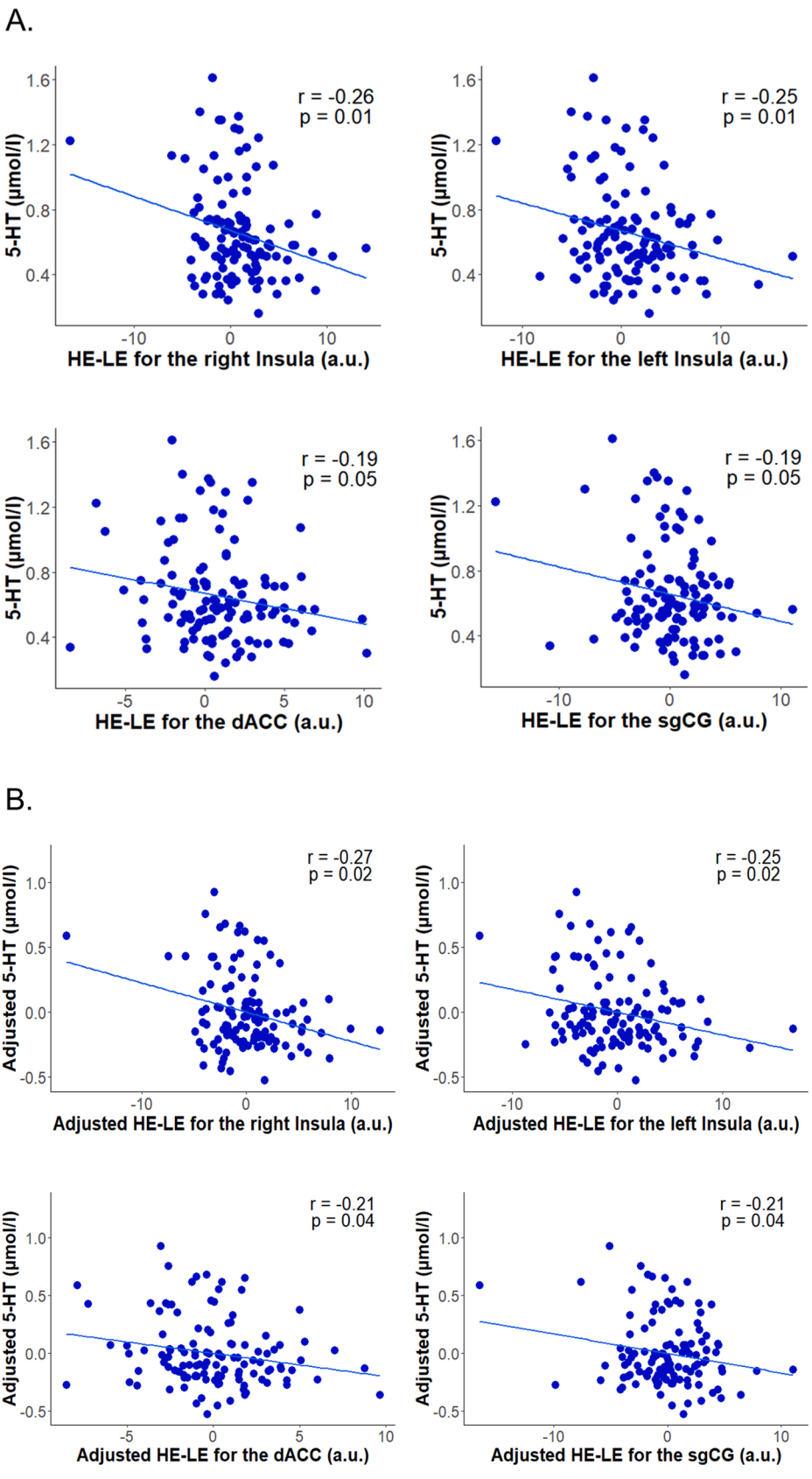

Fig. 3. ROI-based associations between whole-blood serotonin (5-HT) and brain responses to emotional videos (High Emotion – Low Emotion). **A.** Scatter plots depict the significant Pearson's zero-order correlations between whole blood 5-HT levels (in µmol/l, in the Y axis) and brain responses (in arbitrary units, in the X axis) of bilateral insula (upper part), subgenual cingulate gyrus (sgCG) and dorsal anterior cingulate cortex (dACC; lower part). **B.** Scatter plots depict the significant Pearson's partial correlations (adjusted for age and sex) between whole blood 5-HT levels (in µmol/l, in the Y axis) and brain responses (in arbitrary units, in the X axis) of bilateral insula (upper part), subgenual cingulate gyrus (sgCG) and dorsal anterior cingulate cortex (dACC; lower part). All p-values are FDR-corrected for multiple comparisons.

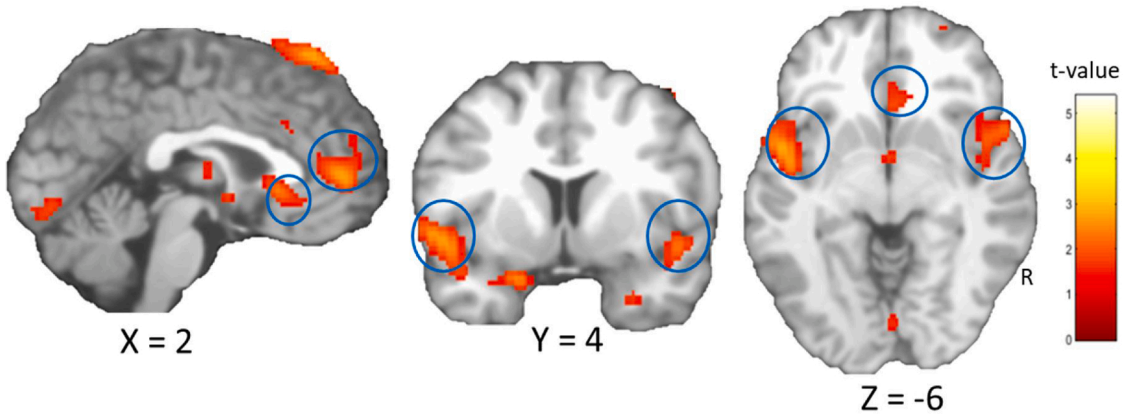

Fig. 4. Whole-brain group-level analyses using the whole-blood 5-HT levels as a covariate. Images are in neurological orientation. T-maps ($p < .001$, uncorrected, $k = 10$) confirm the specificity of our results by showing a negative modulation of the HE—LE contrast in our ROIs (within blue circles) (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Table 3
Relationships between whole blood 5-HT levels and BOLD activity during HE-LE videos and subsequent resting periods in ROIs.

	ROIs	5-hydroxytryptamine (5-HT)	
	Zero- orderPearson correlations	PartialPearson correlations	
HE-LE	Right amygdala	$r = -.08$ $p = .37$	$r = -.08$ $p = .47$
V	Left amygdala	$r = -.14$ $p = .14$	$r = -.14$ $p = .17$
I	Right insula	$r = -.26$ $p = .01$	$r = -.27$ $p = .02$
D	Left insula	$r = -.25$ $p = .01$	$r = -.25$ $p = .02$
E	dACC	$r = -.19$ $p = .05$	$r = -.21$ $p = .04$
O	sgCG	$r = -.19$ $p = .05$	$r = -.21$ $p = .04$
S			
HE-LE	Right amygdala	$r = .04$ $p = .82$	$r = .02$ $p = .99$
R	Left amygdala	$r = .20$ $p = .17$	$r = .20$ $p = .20$
E	Right insula	$r = .04$ $p = .82$	$r = .03$ $p = .99$
S	Left insula	$r = .09$ $p = .82$	$r = .08$ $p = .99$
T	dACC	$r = -.02$ $p = .82$	$r = -.01$ $p = .99$
	sgCG	$r = -.07$ $p = .82$	$r = -.06$ $p = .99$

Pearson's zero-order correlation and partial correlation, adjusted for age and sex.
HE – LE = High Emotion – Low Emotion.
ROIs = Regions of Interest; dACC = Dorsal anterior cingulate cortex; sgCG = subgenual cingulate gyrus.
All reported *p-values* are FDR-corrected for multiple comparisons at $p < 0.05$.

Heightened activity in brain regions particularly sensitive to emotional intense stimuli accords with their putative functional interactions with the serotonergic system (Oathes et al., 2015; Santangelo et al., 2019). Here, we showed a negative correlation between whole blood 5-HT and a differential HE-LE response during videos for the bilateral insula of older individuals, indicating that serotonergic regulation also plays a regulatory role in affective functions in this cohort. This result mirrors the findings of Simmons and colleagues, who showed a diminished activity of the bilateral insula during affective anticipation after increasing synaptic 5-HT availability (i.e., via SSRIs) in healthy young volunteers (Simmons et al., 2009). Similarly, a PET study found that lower 5-HT transporter availability in the insula predicted enhanced fear responses in a healthy sample (Ahs, Frick, Furmark, & Fredrikson, 2015). Critically, variations in the 5-HT transporter gene (i.e., the short allele S of the 5-HTTLPR) seem to modulate the hyper-reactivity of the insula to emotional stimuli in negative self-reflection and pathological anxiety (Klumpp et al., 2014; Ma et al., 2014), lending credence to the idea of diminished serotonergic neurotransmission underlying hyper-vigilant and depressive states (Oathes et al., 2015). Nevertheless, as most studies to date focus on younger participants, future work is still needed to directly address age-dependent effects of the abovementioned results as well as their interaction with circulating 5-HT levels.

We also observed a negative correlation between whole blood 5-HT and differential HE-LE responses for the dorsal anterior cingulate cortex (dACC) during videos, which became statistically significant after controlling for the variance explained by age and sex. Together with the insula, the dACC is a key node of the salience detection network (Seeley et al., 2007). In this line, prior work reported heightened neural responses of this area in response to negative stimuli (i.e., fearful faces), resulting from diminished 5-HT availability for neurotransmission (Carlisi & Robinson, 2018; Robinson et al., 2013) and decreased dACC activation during aversive anticipation, after SSRI administration (Simmons et al., 2009). Furthermore, it has been proposed that 5-HT is implicated in the inhibition of the so-called "aversive amplification" circuit (i.e., prefrontal-amygdala; (Carlisi & Robinson, 2018)), which regulates negative affective bias and exhibits dysfunctional activity in mood and anxiety disorders (Robinson et al., 2013). Here, we only observed an association between whole blood 5-HT and dACC activity, while the correlation between 5-HT levels and amygdalae responses is positive and failed to reach statistical significance. It is possible that, even in situations of low 5-HT levels, healthy individuals might still adequately regulate the aversive amplification circuit mediated by the dACC, thus exerting more frontal control over emotionally induced brain responses with no resulting increases in amygdalar activity. Since the dACC is involved in the executive dysfunction observed in depressed elderly (i.e., depression executive dysfunction syndrome - DED), changes in dACC activity and connectivity have already been reported in the context of geriatric depression (see Alexopoulos (2019) for a review). However, the role of 5-HT in the modulation of both the dACC and the amygdalae should be considered in more detail in the elderly population.

Lastly, we observed that the HE-LE response in sgCG during videos was also negatively correlated with whole blood 5-HT levels. A comprehensive review by Drevets and colleagues drew attention to the pivotal role of this structure in the regulation of emotional behavior, as well as its involvement in motivational and interoceptive changes observed in mood disorders (Drevets et al., 2008). Particularly relevant

for our current results, the sgCG (and its extended area, the subcallosal cortex) displays a dense innervation of 5-HT transporters and 5-HT1A receptors (Varnas et al., 2004), which might confer a high susceptibility to different 5-HT levels (Deza-Araujo et al., 2019; Hornboll et al., 2018). Along these lines of reasoning, it is not surprising that our videos displaying sadness and suffering, elicited more negative affect which might be associated with the functional brain responses of the sgCG, particularly in seniors with lower 5-HT levels.

As outlined above, HE videos elicited more negative affect and empathic responses than LE videos. However, neither these ratings nor the other measures of emotion were correlated with whole blood 5-HT levels. We surmise that this lack of results in the domain of self-reports might be due to the healthy state of our sample, perhaps indicating psychological resilience established through the optimization of particular emotion regulation strategies across their lifespan (Gurera & Isaacowitz, 2019). Indeed, an extensive body of literature has shown that healthy elderly exhibit greater clarity of emotions, which leads to higher emotional stability despite a loss of internal and external resources (Gurera & Isaacowitz, 2019; Urry & Gross, 2010). Hence, emotional brain responses observed in the present study presumably implied only transient changes in the socio-affective state of our participants, but did not constitute a direct threat to their mental wellbeing. In support of this notion, we observed that the negative relationship between whole blood 5-HT levels and brain activity in the ROIs during HE-LE videos was not sustained during subsequent resting periods. Even though a related whole-brain study on the same data set showed lasting carryover effects from video to rest periods in corticolimbic brain circuits (Baez-Lugo et al., 2021), the present results show no relation between activations in ROIs at rest and whole blood 5-HT levels.

Exploratory analyses showed that activity in bilateral amygdala during HE-LE videos correlated positively with scores of anxiety and rumination. At the same time, brain activity after HE-LE videos at rest in both insula and amygdala correlated with self-reports of empathy. As these ROI-behavior associations are purely descriptive and have not been corrected for multiple comparisons, they should be interpreted with caution. More studies are needed to investigate the role of the amygdala, which has been implicated in relevance detection (Sander, Grafman, & Zalla, 2003) and the insula, which is key for salience and interoception (Craig, 2002; Seeley et al., 2007).

Taken together, to the best of our knowledge, our study provides for the first time a compelling description of the functional relationship between an endogenous modulator of affect and brain activity to emotional stimuli in the ageing brain, which may have important implications for better understanding and assessing emotion regulation in both health and disease.

4.1. Limitations

There are some limitations to this study. Whole blood 5-HT is an accessible measure of the availability of circulating 5-HT and may serve as a peripheral proxy for central 5-HT serotonin (Cleare, 1997; Collins et al., 2013). However, we are aware that 5-HT concentration in the blood do not necessarily reflect 5-HT concentrations in the brain and cannot provide information about the availability of other components of the serotonergic system such as transporters and receptors. Furthermore, a single measure of whole blood 5-HT levels may be influenced by long-term diet or inter-individual differences in 5-HT metabolism and stores (Silber & Schmitt, 2010). As such, caution is warranted in the interpretation of our results. In addition, a direct association between neurotransmitter availability and functional brain responses has not yet been established. Even though our methodology follows previous work and our findings converge with similar results obtained after tryptophan or other serotonergic interventions, the correlations observed here do not allow claiming any mechanistic causality between 5-HT levels and functional brain activations to emotional stimuli. Moreover, in the absence of a younger control sample, we cannot show any specific age-dependent effects of 5-HT neuromodulation on emotion processing and brain activity. Hence, our study offers a first descriptive approach to the association of 5-HT with emotional brain functions that should be extended by future longitudinal and cross-sectional studies. Lastly, our findings are restricted to the characteristics of our sample (i.e., healthy older individuals), thus precluding direct inferences to Alzheimer or older depressed patients.

4.2. Conclusion

Aiming to assess the potential influence of 5-HT in the control of socio-affective processes in the ageing brain, our study demonstrated that whole blood 5-HT is associated with stronger brain responses to emotional stimuli in healthy elderly. Specifically, functional responses of emotion-related brain regions engaged by videos portraying suffering people, were negatively correlated with whole blood 5-HT levels. Interestingly, these effects were only present during the videos, but not in the subsequent resting periods, and were not associated with self-report measures of socio-affective functions. Our functional findings mirror pharmacological and genetic studies showing that changes in the serotonergic system may lead to enhanced emotionality with corresponding changes at the brain level. Overall, our results extend previous findings on the link between 5-HT and brain reactivity observed in young individuals, and open new avenues for future work to understand the role of endogenous modulators in emotion processing of older adults.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Yacila I. Deza-Araujo: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Visualization, Writing - original draft. **Sebastian Baez-Lugo:** Methodology, Validation, Formal analysis, Data curation, Visualization, Writing - review & editing. **Patrik Vuilleumier:** Conceptualization, Investigation, Supervision, Project administration, Writing - review & editing. **Anne Chocat:** Methodology, Formal analysis, Investigation, Data curation. **Gaël Chételat:** Methodology, Investigation, Resources, Supervision, Project administration, Writing - review & editing. **Géraldine Poisnel:** Conceptualization, Investigation, Resources, Project administration, Writing - original draft, Funding acquisition. **Olga M. Klimecki:** Conceptualization, Investigation, Writing - review & editing, Supervision, Project administration, Funding acquisition.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.biopsycho.2021.108051.

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