Neurobiology of Aging 73 (2019) 177–189

Contents lists available at ScienceDirect

Neurobiology of Aging

journal homepage: www.elsevier.com/locate/neuaging

# Age-related differences in event-related potentials and pupillary responses in cued reaction time tasks

Maria J. Ribeiro [a,b,c,*], Miguel Castelo-Branco [a,b,c]

[a] CIBIT, Institute for Nuclear Sciences Applied to Health (ICNAS), University of Coimbra, Coimbra, Portugal
[b] CNC.IBILI, University of Coimbra, Coimbra, Portugal
[c] Visual Neuroscience Laboratory, Institute for Biomedical Imaging and Life Sciences (IBILI), Faculty of Medicine, University of Coimbra, Coimbra, Portugal

ARTICLE INFO

*Article history:*
Received 12 April 2018
Received in revised form 18 September 2018
Accepted 22 September 2018
Available online 27 September 2018

*Keywords:*
Pupillometry
ERPs
Contingent negative variation (CNV)
Aging
Reaction time
Noradrenaline

ABSTRACT

Deficits in the noradrenergic system are associated with age-related cognitive decline, yet how healthy aging influences the functional properties of this arousal system is still poorly understood. We addressed this question in humans using pupillometry, a well-established indicator of activity levels in the locus coeruleus (LC), the main noradrenergic center in the brain. We recorded the pupillogram and the electroencephalogram of 36 young and 39 older adults, while they were engaged in cued reaction time tasks known to elicit LC responses in monkeys. Event-related potentials (ERPs) revealed significant group differences. Older adults showed higher cortical activation during preparatory processes reflected in enhanced cue-evoked frontocentral ERPs and reduced parietal ERPs at the time of the motor response. In contrast, the amplitude of the task-related pupillary responses did not show a significant group effect. Our findings suggest that aging-related changes in cortical processing during motor preparation and execution, as documented by electroencephalogram, are not accompanied by changes in the amplitude of activation of the LC, as documented by pupillography.

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## 1. Introduction

Aging is the major risk factor for dementia (Niccoli and Partridge, 2012; Wyss-Coray, 2016). A major challenge has been to identify what changes with age that facilitates the development of cognitive decline and ultimately dementia. One prominent hypothesis proposes that the noradrenergic system plays a protective role against neurodegeneration and cognitive decline (Mather and Harley, 2016; Robertson, 2013). A decline of noradrenergic function in the brain of older individuals might thus contribute to the increased incidence of neurodegeneration in these individuals (Wilson et al., 2013).

Noradrenaline is mostly produced in the small brainstem nucleus, the locus coeruleus (LC), and released throughout the central nervous system (Aston-Jones and Cohen, 2005). Noradrenaline acts as a neuromodulator by changing how the neurons respond to incoming input, increasing cortical excitability (Plewnia et al., 2002), enhancing functional connectivity (Eldar et al., 2013), and enhancing the responses to perceptually salient stimuli while inhibiting the representations of unimportant distractors (Mather and Harley, 2016). Pharmacological manipulations of the noradrenergic system suggest a role in processing speed (Grefkes et al., 2010; Halliday et al., 1989; Nieuwenhuis et al., 2007), temporal and spatial attention (Brown et al., 2016; Coull et al., 2001), inhibitory control (Swann et al., 2005, 2013), cognitive flexibility (Alexander et al., 2007; Campbell et al., 2008), and memory (Hurlemann, 2005). LC neurons fire phasically in response to behaviorally relevant stimuli and are closely coupled in time with motor responses (Clayton, 2004). This phasic activation results in a system-wide increase in responsiveness that is thought to modulate stimulus processing, response decision, and memory formation (Clewett et al., 2018; de Gee et al., 2017). The LC is also involved in the regulation of arousal and autonomic function (Samuels and Szabadi, 2008).

Several lines of evidence suggest that LC structure and function change with aging. In humans, age-related cell loss in the LC has been documented (Manaye et al., 1995), although not all studies have consistently found a significant decrease (Kubis et al., 2000). In rats, the innervation of the hippocampus dentate gyrus and frontal cortex by LC neurons shows an age-dependent decrease (Ishida et al., 2000). Intriguingly, in humans, plasma levels of

* Corresponding author at: Institute for Biomedical Imaging and Life Sciences (IBILI), Faculty of Medicine, University of Coimbra, Polo III - Health Sciences Campus, Azinhaga Santa Comba, Celas, 3000-548 Coimbra, Portugal. Tel.: +351 239480088; fax: +351 239481106.
*E-mail address:* mjribeiro@fmed.uc.pt (M.J. Ribeiro).

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https://doi.org/10.1016/j.neurobiolaging.2018.09.028

noradrenaline increase with aging (Featherstone et al., 1987; Kaplon et al., 2011; Wang et al., 2013), and this increase correlates with poor cognitive function (Wang et al., 2013). LC magnetic resonance imaging (MRI) contrast, as measured with the neuromelanin-sensitive high-resolution T1-turbo spin echo MRI sequence, also increases with age (Clewett et al., 2015; Shibata et al., 2006). Older adults with higher LC neuromelanin MR contrast have higher levels of a measure of cognitive reserve (Clewett et al., 2015) and better memory performance (Hämmerer et al., 2018). However, it remains unclear how age-related differences in MR contrast relate to LC function. Functional neuroimaging of the LC in humans is challenging (Brooks et al., 2013), and, to our knowledge, no study so far has investigated age differences in task-related LC activation using functional MRI. An alternative way of inferring changes in activity levels within the LC is by measuring fluctuations in pupil diameter under isoluminant conditions. Pupil size has been shown to be a reliable indicator of activity in neuromodulatory arousal systems including the LC nucleus, in humans (Clewett et al., 2018; de Gee et al., 2017; Murphy et al., 2014) and animal models (Joshi et al., 2016; Reimer et al., 2016). The effect of aging on task-related pupillary responses is still poorly understood. The findings are task dependent. Nevertheless, when differences are found, these point to a reduction in the amplitude of pupillary responses. Pupil dilations elicited by negative emotional images are reduced in older adults; however, this difference depended on task demands, and in the same study, pupillary responses during image recognition were not significantly different across age groups (Hämmerer et al., 2017). Furthermore, it is possible that the differences observed are related to an age-related difference in emotional regulation and not in LC function per se. In a separate working memory study with no emotional content, pupillary responses during encoding showed no age differences, whereas pupillary responses during retrieval were significantly decreased in older adults (Van Gerven et al., 2004). Finally, pupillary responses in search tasks showed no age-related differences (Granholm et al., 2000; Porter et al., 2010). Thus, further studies are warranted to determine which aspects of LC function are affected by aging.

Our main study aim was to determine if task-related activation of the noradrenergic LC nucleus was different in older in comparison with young adults. For this, we used pupillography as an indirect measure of LC activity, as in previous studies [e.g., Hämmerer et al., 2017]. Simultaneously to the acquisition of the pupillogram, we also acquired the electroencephalogram (EEG). The LC sends inputs to most brain regions, with the prefrontal cortex being the cortical region with the highest density of noradrenergic varicosities (Aston-Jones and Waterhouse, 2016; Chandler et al., 2014). In turn, the prefrontal cortex innervates the LC and affects its activity levels. For example, in rats, lesions in the medial prefrontal cortex reduce activity in the LC (Gompf et al., 2010). The dialog between the prefrontal cortex and the LC suggests that the activity in these regions is correlated. Thus, it is possible that age-related changes in cortical function are, at least in part, a consequence of changes in the function of this brainstem noradrenergic system and *vice versa*. In this study, we tested this hypothesis by looking at the relationship between the amplitude of event-related potentials (ERPs) and pupillary signals across participants.

We probed these physiological signals during cued reaction time (RT) tasks with no emotional content known to induce pupillary responses in humans (e.g., Jennings et al., 1998) and phasic LC responses in monkeys (Clayton, 2004). Pupil dilation is commonly observed in cued RT tasks during the preparatory period between cue and target (Jainta, 2011; Jennings et al., 1998; Moresi et al., 2008, 2011; Tona et al., 2016; van der Molen et al., 1989), thus suggesting the involvement of brainstem neuromodulatory arousal systems in neural preparatory processes. Several studies suggest that aging affects neural preparation for incoming events. In comparison with young adults, older adults present deficits in the ability to prepare for a specific motor pattern (Sterr and Dean, 2007) and show reduced modulation of motor cortical excitability during the preparatory period suggesting diminished motor preparation particularly in more demanding task conditions (Duque et al., 2016). Moreover, prefrontal hyperactivation is frequently observed in older adults during motor planning, suggesting the need for compensatory activations (Berchicci et al., 2012; Fernandez-Ruiz et al., 2018; Wild-Wall et al., 2007).

We used auditory tasks to study the pupillary responses elicited by cognitive processes without the interference of luminance-related pupillary changes. We compared across groups and task conditions on a time point-by-time point basis thereby determining with precision and in a data-driven manner, which aspects of task processing presented significant differences across groups. We controlled for multiple comparisons using a bootstrap spatial-temporal clustering technique (Maris and Oostenveld, 2007; Pernet et al., 2015). We studied ERPs and pupillary responses while participants were performing 3 different task conditions: a passive task, involving only the perception of auditory stimuli, and 2 active tasks, a cued simple RT task and a cued go/no-go task. The comparison between the passive and the active tasks allowed us to isolate the pupillary responses related to motor preparatory processes. Our simple RT task required reporting the detection of an auditory stimulus by button press as fast as possible. These types of tasks have been used as measures of processing speed. Our go/no-go task had the characteristics of a choice RT task. It required not only stimulus detection but also stimulus identification (discrimination between 2 different auditory stimuli) followed by response selection (whether to press the button or withhold the response). Reaction time slows down with aging much more on choice tasks than on simple detection tasks (Der and Deary, 2009; Woods et al., 2015a,b; Yordanova et al., 2004). Given the previous findings, we predicted that the RT differences between young and older adults would be more pronounced in the go/no-go task. Pupil dilation and activity in neuromodulatory brainstem nuclei, including the noradrenergic LC, have been linked to decision-making processes (de Gee et al., 2014, 2017). An interesting question we sought to address was to investigate how task-related pupillary responses differ across tasks requiring different levels of decision complexity by comparing 2 age groups that we predicted would present performance differences. We expected pupil dilation to present higher amplitude in the go/no-go condition, where the decision complexity was highest. Moreover, we expected age-related differences in pupil dilation to reflect differences in task performance with older individuals failing to increase their arousal to fully meet the demands of the go/no-go task. These hypothesized group differences in arousal modulation might underlie, at least in part, the age-related difficulty previously identified in choice tasks.

In cued RT tasks, the cue alerts the participant and elicits preparatory processes, namely, orientation of attention toward the anticipated target stimulus, preparation of the anticipated motor response, and executive control to maintain the anticipatory focus. We studied the ERPs and pupillary responses elicited by the cue stimulus, thereby studying engagement of cortical and brainstem arousal systems during these preparatory processes. In addition, we studied the ERPs and pupillary responses locked to target and button press to investigate age differences in the processes related to target detection and discrimination, response selection, and motor output.

## 2. Material and methods

### 2.1. Participants

Thirty-six young adults and 39 older adults were included in this study. All participants had normal hearing and normal or corrected to normal vision; had no history of neurological, psychiatric, or vascular disease; and were not taking any psychotropic medications. Current medication with beta-blockers was an exclusion criterion. Six participants were taking other types of nonadrenergic medication to control blood pressure. Participants were asked to sleep sufficiently the night before the recording and to avoid the use of alcohol. Below the cutoff result in the Montreal Cognitive Assessment—MoCA, a dementia screening tool (Freitas et al., 2011), was also an exclusion criterion. Table 1 shows participants' characteristics.

EEG data from 1 older participant were not included in the analyses due to bad signal quality. Pupil data from 1 young adult and 1 older adult with light colored eyes were not included due to poor data quality.

The study was conducted in accordance with the tenets of the Declaration of Helsinki and was approved by the Ethics Committee of the Faculty of Medicine of the University of Coimbra. Written informed consent was obtained from the participants, after explanation of the nature and possible consequences of the study.

### 2.2. Task design

We designed a cued auditory task consisting of 3 different conditions: a passive listening condition, a cued simple RT condition, and a cued go/no-go condition. The task was designed and run with the Psychophysics Toolbox, version 3 (Brainard, 1997), for Matlab (The MathWorks Company Ltd). The auditory stimuli were single-frequency signals (pure tones) with duration 250 ms, with the following frequencies: cue, 1500 Hz; go stimulus (S1), 1700 Hz; no-go stimulus (S2), 1300 Hz; and error feedback signal, 1000 Hz. The sounds were played at around 67 dB(A) from a hi-fi speakers system (Genius, KYE Systems Corp). All stimuli were suprathreshold.

Participants were requested to fixate a plus sign presented on a gray background, on the center of a computer screen (19-inch Dell monitor), set to 60 Hz refresh rate. The luminance of the background was 43 cd/m$^2$, and the luminance of the fixation cross was 58 cd/m$^2$. Participants were also requested to minimize blinking rate.

A schematic representation of task design is presented in Fig. 1. The experiment started with the passive listening condition containing 30 trials and lasting around 4 minutes. In this condition, participants were instructed to fixate their gaze on the plus sign displayed on the center of a computer screen and to pay attention to the sounds being presented without any other overt task. This condition allowed us to isolate the effect of auditory processing from motor preparatory processes in task-related pupillary responses. After a self-paced break, participants performed the 2 active tasks: cued simple RT and cued go/no-go. The order of these 2 conditions was counterbalanced across participants, that is, half of the participants started with the simple RT and the other half with the go/no-go. Each task was divided in two 8-minute runs with self-paced breaks between runs and between tasks. Just before starting each task, the rules of the task were explained and participants engaged in a practice run ensuring he/she understood the rules and was able to detect and discriminate the different sounds. In the simple RT condition, the cue was followed by a go stimulus (S1: 100 trials) to which participants were instructed to respond by pressing a button on the keyboard as fast as possible with their right index finger. In 17 % of the trials, distributed pseudorandomly throughout the run, the go stimulus was not presented and no response was required (cue-only trials: 20 trials). In the go/no-go task, the cue was followed or by the go stimulus (S1: 80 trials) or by the no-go stimulus (S2: 20 trials). Participants were instructed to respond as fast as possible to the go stimuli with their right index finger, while refraining from responding to the no-go stimuli. Cue-only trials were also included in this condition (20 trials). Total number of trials in each condition was 120. To ensure fast responses in both task conditions, slow responses (RT slower than 700 ms) were signaled with a feedback tone presented 1200 ms after the go stimulus. The aim of signaling slow trials was to apply time pressure (Mückschel et al., 2017).

We considered as error trials all trials where the participants responded after cue presentation, failed to respond to the go stimulus (misses), responded to the go stimulus too slowly (slower than 700 ms), or responded to the no-go stimulus in the go/no-go condition. These trials were signaled with a feedback tone warning the participants that an error was committed. The tone was presented 200 ms after the button press when participants responded between cue and target and 1200 ms after the target in slow trials or responses to no-go.

The intertrial interval was quite long, with a median of 7.6 seconds (min 6.7 and max 19.6 seconds), to ensure pupil diameter returned to baseline values before the beginning of the next trial. The interval between the cue and the target stimuli and between target and the beginning of the next trial were drawn from a nonaging distribution, $-W^*\ln(P)$, where W is the mean value of the interval distribution and P is a random number between 0 and 1. In our task design, the cue-target interval was $1.5-0.25^*\ln(P)$ in seconds, and the interval between the target and the beginning of the next trial (cue) was $5.2-1^*\ln(P)$ in seconds.

### 2.3. Behavioral analysis

For the RT analysis, we included all correctly responded trials including trials where the response time surpassed 700 ms but excluded the correct trials that immediately followed error trials, so that trials affected most by the arousing caused by the error awareness and feedback stimulus were not included.

Statistical analysis of RT data was performed using IBM SPSS Statistics, version 19 software. We used repeated-measures analysis of variance (ANOVA) with task conditions (simple RT and go/no-go) as a within-subject factor and group as a between-subject factor. Comparison of number of errors was performed using nonparametric statistics Mann-Whitney.

**Table 1**
Participants' characteristics

| | N | Age (y) | | | Sex | | Education (y) | | | MoCA scores | | | Handedness | | Smokers |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | Min | Max | F | M | Mean | Min | Max | Mean | Min | Max | Left | Right | |
| Young | 36 | 23 | 19 | 30 | 29 | 7 | 16 | 12 | 21 | 27 | 22 | 30 | 3 | 33 | 3 |
| Older | 39 | 60 | 52 | 70 | 31 | 8 | 16 | 4 | 23 | 25 | 18 | 30 | 3 | 36 | 9 |

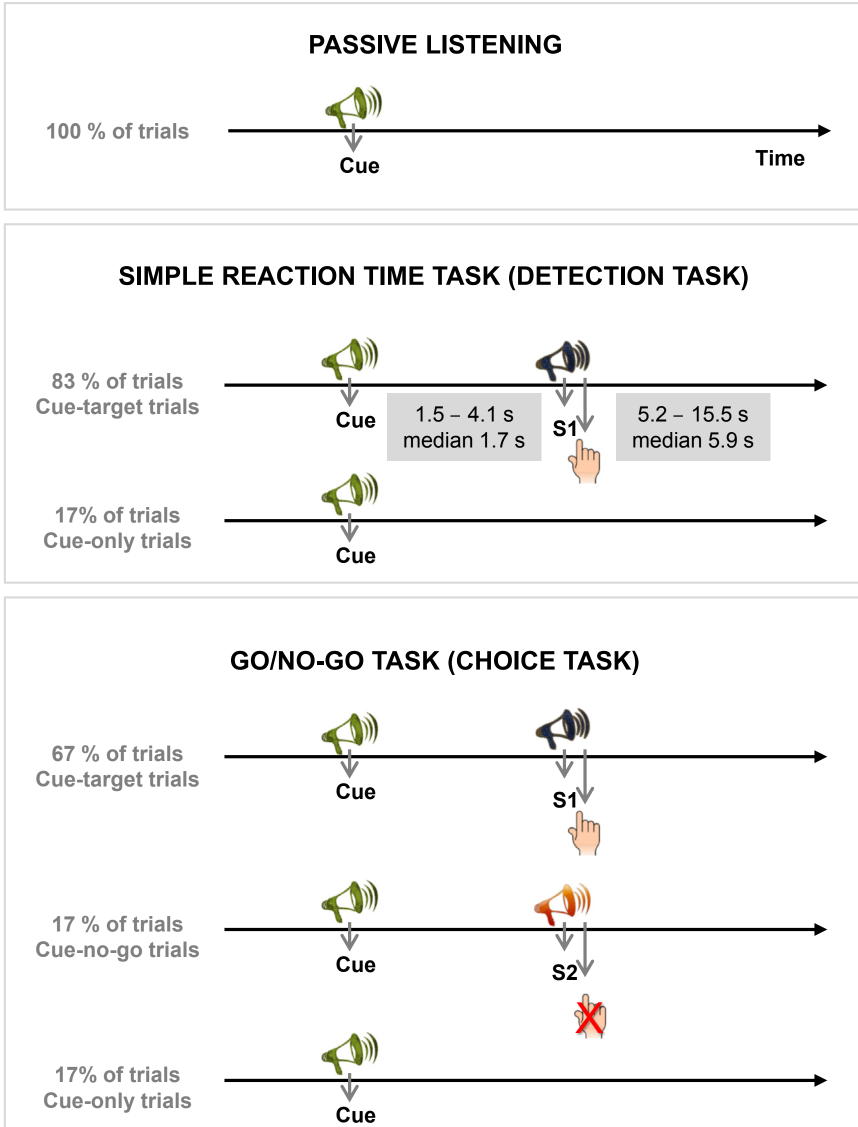


**Fig. 1.** Schematic representation of the task design.

### *2.4. EEG acquisition and analysis*

EEG signal was recorded using a 64-channel Neuroscan system with scalp electrodes placed according to the International 10–20 electrode placement standard, with reference between the electrodes CPz and Cz and ground between FPz and Fz. Acquisition rate was 500 Hz. Vertical and horizontal electrooculograms were recorded to monitor eye movements and blinks. Bipolar electrocardiogram (ECG) electrodes were placed on the chest. The ECG data analysis will be presented elsewhere. During data acquisition, the participants' head was stabilized with a chin and forehead rest. Consequently, the electrodes on the forehead, FP1, FPz, and FP2, displayed signal fluctuation artifacts due to the pressure on the forehead rest. These were excluded from the analyses. A trigger pulse was generated at the onset of each stimulus and at every button press. Electrode positions were measured using a 3D-digitizer Fastrak (Polhemus, VT, USA). EEG data analysis was performed with the EEGLAB toolbox, version 14.1.1 (Delorme and Makeig, 2004).

We used independent component analysis (ICA) to eliminate nonbrain artifacts from the data. For this, the EEG data were re-referenced to linked earlobes and band-pass filtered using the Hamming windowed sinc FIR filter with cutoff frequencies of 1 and 100 Hz; bad channels were rejected; the continuous data were visually inspected, and segments containing considerable artifacts were removed; finally, for each participant, the data from all runs independent of the condition were merged. The merged data sets were then submitted to extended Infomax ICA (Bell and Sejnowski, 1995) to find components associated with nonbrain artifacts. We used the default parameters of the binica program in EEGLAB. The independent components (ICs) activity data were cut into segments locked with the cue onset from −700 ms until 6000 ms, and average baseline activity (set from −200 ms to cue onset) was removed from each trial. Next, we inspected the spatial, spectral, and temporal properties of each IC to identify those components corresponding to non-brain sources: eye blinks, lateral eye movements, muscular artifacts, single-channel artifacts, and high-frequency line noise. The ICA matrix was then imported to the original continuous data runs, re-referenced to linked earlobes, and with bad channels removed. The artifact ICs were excluded from further analyses. The data were then band-pass filtered with cutoff frequencies of 0.1 and 35 Hz, and periods containing further artifacts were manually removed. Data were cut into segments locked with the cue onset from −200 ms until 6000 ms after cue onset, and average baseline activity (set from −200 ms to cue onset) was removed from each trial. Bad channels were then interpolated.

The number of trials included in the analyses after artifact rejection did not differ significantly across age groups ($p > 0.5$; Supplementary Table 1).

Statistical analyses were conducted using the LIMO EEG toolbox (Pernet et al., 2011), which models the data across trials at all time points and all electrodes using a hierarchical approach. First, data are modeled across all trials using a general linear model at the participant level, and then first-level parameter estimates are tested at the group level across participants. We controlled for multiple comparisons using a bootstrap spatial-temporal clustering technique (Maris and Oostenveld, 2007; Pernet et al., 2015). First-level analyses (single-subject level) were performed using the LIMO EEG batch mode running PSOM (Bellec et al., 2012). The model included task conditions (simple RT and go/no-go) as categorical predictors (design matrix in Supplementary Fig. 1A). Parameter estimates were computed using single-trial ordinary least squares. In the cue-locked analyses, parameter estimates were computed from cue onset to 3000 ms after. In the target-locked analyses, parameters were computed from −200 ms to 1500 ms after target onset. In the response-locked analyses, parameters were computed from −500 ms up to 1000 ms after button press. For each participant, we obtained for every electrode the time course of model parameters. At the group level (second-level analysis), we compared the first-level parameter estimates using a repeated-measures ANOVA with task type as a within-subject factor and age group as a between-subject factor. The model had thus 2 categorical predictors, task type and group, and an interaction term between task conditions and group.

### 2.5. Pupillography data acquisition and analysis

The pupil diameter of the right eye was measured by an infrared eye-tracker (iView X Hi-Speed 1250 system from SMI) with a sampling rate of 240 Hz. A trigger pulse was generated at the onset of each stimulus and at every button press.

Analysis of pupil data was performed using custom scripts and the EEGLAB toolbox in Matlab R2015a and R2017b. Artifacts and blinks were corrected using the blink correction Matlab script by Greg Siegle (stublinks.m) available in https://www.pitt.edu/~gsiegle/ (Siegle et al., 2003, 2008). Briefly, artifacts, including blinks, were identified as large changes in pupil dilation occurring too rapidly to signify actual dilation or contraction. Linear interpolations replaced artifacts throughout the data sets. Data were smoothed using a 3-point unweighted average filter applied twice. After automatic artifact correction, pupil data were imported into EEGLAB and cut into epochs locked to cue onset from −1000 until 6000 ms. Pre-cue pupil diameter (pupil baseline), measured as the average dilation over the 200 ms preceding the onset of the cue, was subtracted from the pupil diameter values to produce task-related pupil dilation responses. Pupil epochs were visually inspected for artifacts not adequately corrected by the linear interpolation procedure. Epochs with remaining artifacts were manually rejected. In addition, we discarded all error trials and correct trials that immediately followed error trials. The numbers of included trials did not differ significantly across groups for each condition (Supplementary Table 1).

As has been observed before (Moresi et al., 2008), in both groups of participants, the amplitude of the task-related pupillary responses elicited by the cue was associated with average pre-cue pupil diameter, in a way that individuals with on average bigger pupils produced task-related pupillary responses with higher amplitudes, that is, the physiological response increased as a function of baseline (Supplementary Fig. 2A). Thus, to obtain a measurement of task-related pupillary responses independent of average pupil size, we normalized the pupillary responses by transforming pupil diameter into percent dilation, according to the following formula (pupil diameter − pre-cue pupil diameter)/pre-cue pupil diameter * 100. Using this measurement, the amplitude of the task-related responses and pupil baseline were no longer correlated (Supplementary Fig. 2B). This normalization was applied before by Moresi et al. (2008).

As for the EEG data, statistical analyses were conducted using the LIMO EEG toolbox (Pernet et al., 2011). This toolbox allowed us to model pupil data using a hierarchical GLM across trials at all time points. First, data were modeled across all trials using a general linear model at the subject level, and then first-level parameter estimates were tested at the group level across participants. We controlled for multiple comparisons using a bootstrap temporal clustering technique (Maris and Oostenveld, 2007; Pernet et al., 2015). To statistically compare the task-related pupillary responses elicited in both groups of participants in the different task conditions, we used a linear hierarchical model, where, in the first level, we modeled the effect of task at the single-subject level, and, in the second level, we studied group effects by comparing the predictors obtained in the first level. In the analysis comparing correct go trials across task conditions and age groups, we compared the parameters obtained from the single participants linear model using repeated-measures ANOVAs with task condition as a within-subject factor (simple RT vs go/no-go) and age group as a between-subject factor (young vs older adults). In cue-locked data, these analyses were done for each time point of the pupillary response in a 6-second time window starting at cue onset, in target-locked data, the time window analyzed was between −1500 and 2500 ms locked with target onset, and, in response-locked data, the analyses were performed in a time window between −1400 and 2400 ms after button press. In the analysis comparing go and no-go trials within the go/no-go task condition, we compared the parameters obtained from the single participants linear model using repeated-measures ANOVAs with trial type as a within-subject factor (go vs no/go) and age group as a between-subject factor (young vs older adults). For this analysis, to equalize the number of trials used for each trial type (go and no-go), for each participant, we randomly selected go trials up to the same number of the no-go trials included (Supplementary Table 1).

As a control analysis, we compared blink frequency across groups within the cue-locked epochs of the passive task and correct go epochs of simple RT and go/no-go tasks. This was done on a time point-by-time point basis allowing us to study how many epochs contained blinks at each time point within the cue-locked epoch, that is, blink frequency per time point. In line with the findings from Siegle et al. (2008), we observed that, for both groups of participants and in both active task conditions (simple RT and go/no-go), blinks occurred more often in the period after the cue, and at the end of the trial after the motor response. In turn, blinks were suppressed to below baseline levels before target onset (Supplementary Fig. 3). For each time point, blinks occurred on average in less than 10% of the trials. In the go/no-go task, the older group presented a tendency to blink sooner after the motor responses. This difference was small and represented an increase of less than 5% of the trials at any time point. Moreover, time point-by-time point $t$-test comparison across groups did not reveal any significant group difference in the percentage of trials with blinks for all task conditions after controlling for multiple comparisons using the bootstrapping technique and temporal clustering correction with LIMO EEG.

Therefore, any group difference in pupillary responses is unlikely to be a result of differences in blink rate.

## 3. Results

### 3.1. Behavioral results

Reaction time analysis revealed a significant effect of task conditions [simple RT vs. go/no-go: $F_{(1, 73)} = 155$, $p < 0.001$] with correct responses in the go/no-go condition significantly slower than the correct responses in the simple RT condition (Fig. 2). In addition, the statistical analysis revealed a significant Task × Group interaction [$F_{(1, 73)} = 18.5$, $p < 0.001$] and no effect of group [$F_{(1, 71)} = 0.016$, $p = 0.900$]. The significant interaction results from the fact that the difference in reaction times between conditions was higher in the older group than in the young group. Indeed, the older participants, in comparison with young participants, responded on average faster, in the simple RT task that required tone detection but responded slower in the go/no-go condition that required tone discrimination.

To evaluate task accuracy, we calculated the number of errors, including missed go trials, responses to the cue, and responses to the no-go stimulus in the go/no-go condition. Numbers of errors were very low. Participants committed on average 2 errors in the simple RT condition and 4 errors in the go/no-go condition. Misses and responses to the cue occurred more often in the older group than in the young group. Mann-Whitney comparisons on the number of errors per group revealed a significant effect of group, in both task conditions, in the number of misses ($p < 0.05$; simple RT: mean ± SD young group = 0.17 ± 0.38, older group = 0.82 ± 1.25; go/no-go: mean ± SD young group = 0.22 ± 0.42, older group = 1.15 ± 2.16) and responses to the cue ($p < 0.05$; simple RT: mean ± SD young group = 0.42 ± 0.69, older group = 1.10 ± 1.33; go/no-go: mean ± SD young group = 0.14 ± 0.35, older group = 0.64 ± 1.33). In contrast, the number of button presses to the no-go stimulus was not significantly different across groups ($p = 0.400$; mean ± SD young group = 0.94 ± 0.95, older group = 1.49 ± 1.90).

### 3.2. Event-related potentials

We analyzed the cue-, target-, and response-locked ERPs of both groups of participants while they were engaged in the simple RT and go/no-go tasks. We restricted this analysis to correct go trials. Given that task-related activity started with the onset of the cue, in the analysis of ERPs locked to target onset and to button press, we kept the baseline before cue onset, that is, just before the start of the trial.

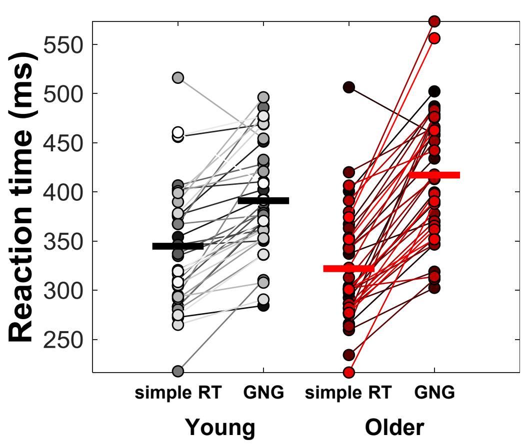


**Fig. 2.** Reaction time values of both groups of participants, for simple reaction time (RT) and go/no-go (GNG) task conditions. Black and red horizontal lines represent mean reaction time values of the young and older group, respectively. Each grey circle represents the median reaction time of each young participant and each red circle represents the median reaction time of each older participant.

The amplitude of the ERPs presented group and task effects across multiple electrodes at several time points both before and after target onset that occurred on average around 1700 ms after cue onset (Fig. 3 and Supplementary Figs. 4 and 5). Between cue and target stimuli, during the preparatory period, group effects reflected differences in the contingent negative variation (CNV), a frontocentral potential that is associated with neural preparatory processes (Di Russo et al., 2017). The older group presented a CNV potential with higher absolute amplitude than the young group, suggesting that the older participants engaged more neural resources in preparation for stimulus processing and motor responses. During the preparatory cue-target interval, we also observed an effect of task (simple RT vs go/no-go), mainly in frontal and central electrodes (Fig. 3A). In the go/no-go task, the absolute amplitude of the CNV potential was higher than in the simple RT task (Fig. 3B and C), suggesting that participants engaged more neural resources in the choice task while preparing for stimulus discrimination than in the simple RT task while preparing for stimulus detection. This task effect was, however, smaller in the older group. Accordingly, a significant Task × Group interaction effect was observed in the analysis of the target-locked ERPs during a time window starting before target onset and finishing soon after, mainly in frontal and central electrodes. Before target onset, the difference across tasks was larger in the young in comparison with the older group (Fig. 4A and Supplementary Fig. 4). In the analysis of the cue-locked ERPs, although there was a trend for a Task × Group interaction (Fig. 3C), this was not significant at any time point after controlling for multiple comparisons.

After target onset, a positive potential was apparent in parieto-occipital electrodes presenting higher amplitude in the young in comparison with the older group, in cue-, target-, and response-locked ERPs (Figs. 3B, C and 4). Effects of task were also observed after target onset where the positive parieto-occipital potential had higher amplitude in the go/no-go than in the simple RT task. In target-locked analyses, the time course of parietal electrodes revealed a positive deflection in the young group peaking around 300 ms after target onset (P300), which amplitude was markedly reduced in the older group, in both task conditions (Fig. 4A). Analyses of the response-locked ERPs suggested that this parietal potential was locked with button press (Fig. 4B) and in the young group presented a sustained activity after the motor response that was absent in the older group. This parietal positivity also presented an effect of task having higher amplitude in the go/no-go task in comparison with the simple RT task, and in the target-locked analysis presented a significant Task × Group interaction reflecting the fact that the task difference was more pronounced in the young in comparison with the older group (Fig. 4 and Supplementary Fig. 4).

In sum, ERP analyses revealed enhanced preparatory cortical activity in older adults, consistent with neural hyperactivation thought to underlie compensatory mechanisms in older individuals (Reuter-Lorenz and Cappell, 2008; Reuter-Lorenz and Park, 2014). Frontocentral preparatory activity was higher in the go/no-go task in comparison with the simple RT task, yet this difference was reduced in the older group, suggesting that the older group did not modulate their preparatory effort according to task demands. Moreover, the older group presented reduced parietal activation following target onset and locked with button press consistent with the reduced P300 amplitude frequently reported in this age group (Dinteren et al., 2014). This parietal positivity presented higher amplitude in the go/no-go task than the simple RT task, yet this task difference was reduced in the older group.

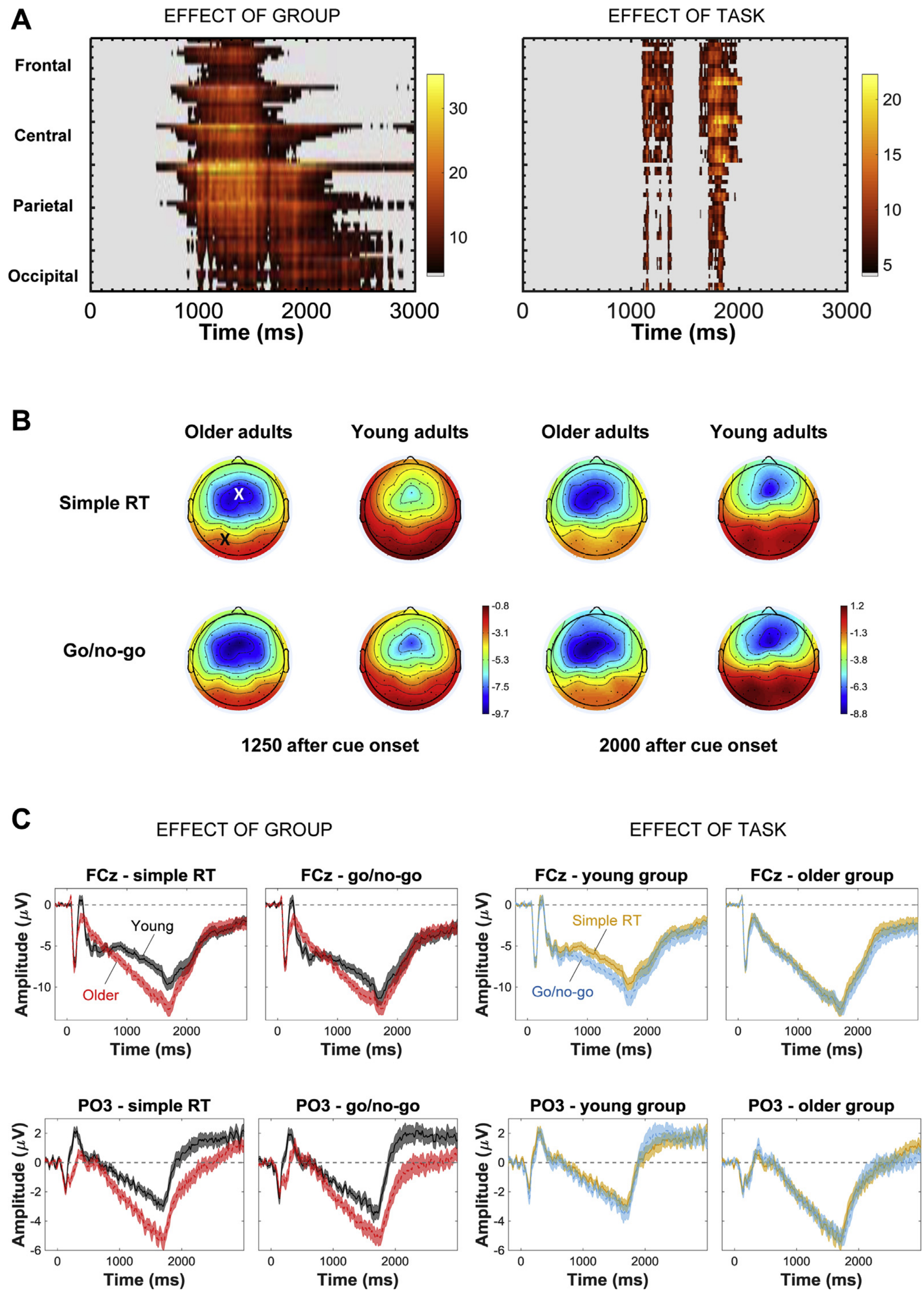


**Fig. 3.** (A) Plot of the repeated-measures ANOVA *F*-values within the time windows where the amplitude of cue-locked ERPs presented statistically significant effects of group and task, after correction for multiple comparisons by the spatial-temporal clustering method. The effects of Task × Group interaction were not significant after correction for multiple comparisons. Electrodes are stacked up along the y-axis. (B) Cue-locked ERPs scalp topography at 1250 ms and 2000 ms after cue-onset. Electrodes FCz and PO3 are marked with white and black Xs, respectively. (C) ERPs at electrodes FCz and PO3 for both task conditions and both groups, in the left, comparing across groups, in the right, comparing across tasks (mean ± standard error across participants). Abbreviations: ANOVA, analysis of variance; ERP, event-related potential; RT, reaction time.

### 3.3. Task-related pupillary responses

Given the sluggishness of the pupillary responses (Joshi et al., 2016), the responses associated with the preparatory processes elicited by the cue stimulus overlap with processes associated with target processing and motor responses. We were able to isolate the pupillary responses associated with preparatory processes from the responses evoked by target and motor responses by including in the task a small percentage of cue-only trials where participants prepared for target processing and for the motor response, but in the absence of target, these processes did not occur. To also separate the pupillary response elicited by the auditory processing of the cue stimulus from the proactive preparatory processes, we compared the cue-only trials of the simple RT and go/no-go tasks with the

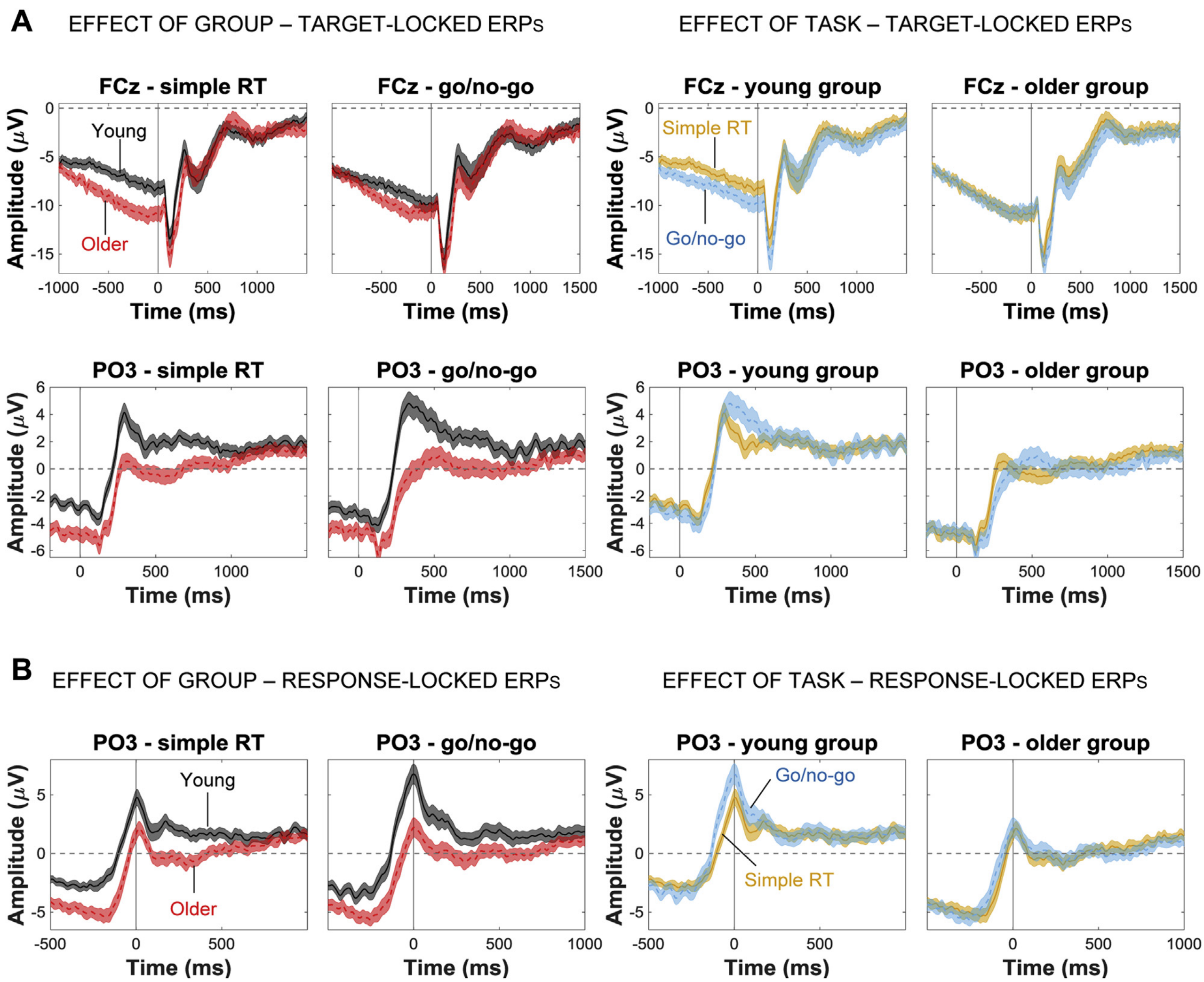


**Fig. 4.** Target-locked (A) and response-locked (B) event-related potential (ERPs) with baseline before cue onset, that is, just before the start of the trial, measured at the fronto-central electrode FCz and at the parieto-occipital electrode PO3. Graphs represent mean ± standard error across participants.

pupillary responses elicited in the passive task, where participants were listening to the same auditory stimulus used as cue in the active tasks without performing any overt task. In the passive task, the auditory tone elicited a small pupil dilation response that peaked around 600 ms after stimulus onset and returned to baseline around 2000 ms after (Fig. 5A). This early pupillary response was present also in cue-only and cue-target trials (Fig. 5A and B), suggesting therefore that the sensory perception of an auditory stimulus evokes a small pupillary dilation. Cue-only trials, in the active simple RT and go/no-go tasks, were perceptually the same as the trials in the passive task except that now the auditory stimulus acted as a temporal cue for the RT tasks. Under these conditions, we observed a second pupillary dilation that peaked around 2700 ms after the cue (Fig. 5A and B). This second pupil dilation must thus be associated with the preparatory processes elicited by the cue. Notably, the pupillary responses in cue-only trials were very similar to the pupillary responses in cue-target trials suggesting that a significant component of the pupillary responses was associated with preparatory processes (Fig. 5A and B). Target onset occurred on average around 1700 ms after the cue. From this time point onwards, pupillary responses elicited by target processing overlap with pupillary responses associated with the preparatory processes elicited by the cue. We studied these processes by looking at target-locked and response-locked pupillary responses maintaining the baseline just before cue onset. In that way, the pupillary response included the response started at cue-onset as well as the response evoked by the target and button press. By locking the pupillary responses to target onset and button press, we were able to define differences in amplitude in relation to these events. Finally, we explored differences across go and no-go trials in the go/no-go task (Fig. 6).

Time point-by-time point statistical comparisons revealed differences across task conditions but no group differences or Task × Group interactions. The time windows presenting significant task effects at the group level are represented in Fig. 5 as horizontal gray bars in the plots displaying the effect of task. For the cue-only trials, we studied the effect of task including the 3 task conditions as within-subject factors (passive, simple RT, and go/no-go) allowing us to determine the time window where the passive task differed from the active tasks. This analysis revealed task differences starting 580 ms after cue onset. To understand how the pupillary responses varied across the 2 active tasks, we studied the effect of task including only the 2 active tasks as within-subject factors (simple RT and go/no-go). We observed an effect of active task in both cue-only trials and cue-target trials, with the go/no-go task eliciting higher pupillary dilations than the simple RT task in both

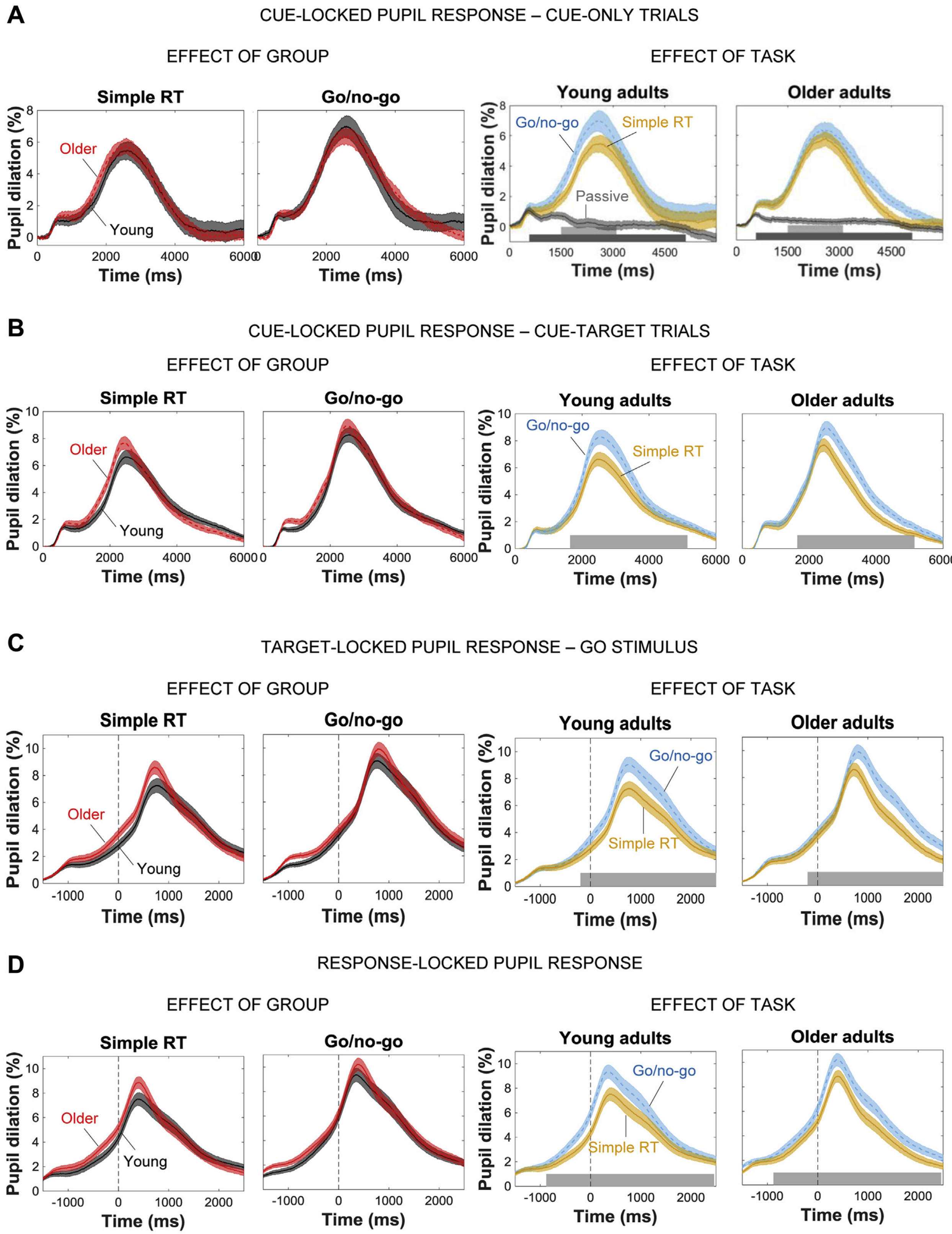


**Fig. 5.** Task-related pupillary responses. (A) Cue-locked pupillary responses of cue-only trials. (B) Cue-locked pupillary responses of correct go trials. Target onset occurred around 1700 ms after cue onset. (C) Target-locked pupillary responses of correct go trials. (D) Response-locked pupillary responses of correct go trials. Graphs represent mean ± standard error across participants. The gray bars indicate the time windows presenting significant task effects at the group level in the time point-by-time point comparison of the task-related pupillary responses. In (A), in the analysis of the cue-only trials, the time window presenting a significant effect of task from the repeated-measures ANOVA including as the within-subject factor the 3 tasks (passive, simple RT, and go/no-go) is represented by the dark gray, bar whereas the time window indicating the significant task effect in the repeated-measures ANOVA including as the within-subject factor only the 2 active tasks (simple RT and go/no-go) is represented by the lighter gray bar.

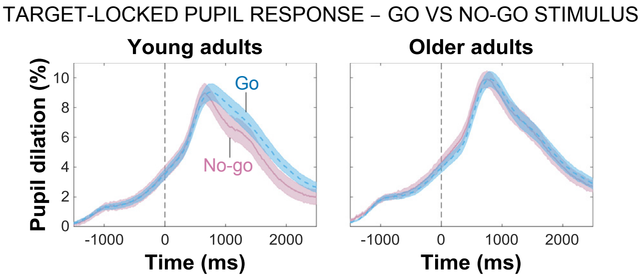


**Fig. 6.** Comparison of target-locked task–related pupillary responses elicited by the go and no-go stimuli in the cued-go/no-go task, in young and older adults. Graphs represent mean ± standard error across participants.

groups of participants (Fig. 5A and B). These observations suggest that, as in the ERP findings, also the pupillary response elicited by the cue presents higher amplitude when associated with the preparation for a choice task than when associated with the preparation for a detection task, that is, it is modulated by the complexity of the decision. The target-locked analysis revealed that the pupillary responses of the 2 task conditions started to differ ~200 ms before target onset further confirming that these conditions differ already during the preparatory period (Fig. 5C). In the response-locked analysis, we observed a significant task effect starting 875 ms before button press and lasting up to 2379 ms after button press (Fig. 5D). Comparison across go and no-go trials in the go/no-go task did not reveal any significant differences suggesting similar pupillary responses in these trials (Fig. 6).

Although comparison across groups of the amplitude of the task-related pupillary responses did not reveal a significant group effect, it is possible that other measures of pupil dynamics would capture a difference. We explored 2 additional types of analyses: the time derivative of the pupillary responses and single trial measurements of peak amplitude and latency. These analyses are briefly presented in Supplementary Material as secondary analyses (see Supplementary pages 6–8) and would need to be explored further in future studies.

## 4. Discussion

ERPs and pupillary responses elicited during the performance of cued auditory RT tasks revealed dissociable aging-related effects. Although our ERP findings showed differences during the preparatory period with older adults showing enhanced frontocentral responses, no group differences were observed in the amplitude of the pupillary responses associated with preparatory processes. During target processing, response decision, and response execution, ERPs revealed reduced parietal activity in older adults. Yet, our time point-by-time point analysis of the amplitude of the pupillary responses did not reveal any group differences associated with these neural processes. Moreover, our ERPs' analyses revealed interactions between group and task that were not observed in the pupillary analyses. These results suggest that age-related changes in cortical processing, observed during the performance of cued RT tasks, are not associated with changes in the activation of arousal systems, as assessed through the amplitude of task-related pupillary responses.

To our knowledge, this was the first study investigating the effect of aging on task-related pupillary responses in RT tasks. Our findings suggest that the amplitude of the pupillary responses in these types of tasks is not affected by aging thereby implying that activation of brainstem arousal systems as assessed by task-related pupillary responses is not generally dampened or enhanced by aging and appears, in older adults, remarkably similar to the activation observed in young adults. Similar observations have been made in previous studies; for example, Granholm et al. (2000) did not find an effect of aging while investigating pupillary responses elicited during a partial-report span of apprehension task requiring fast target detection in the presence of distracters (Granholm et al., 2000). Nevertheless, age-related changes in pupillary responses might emerge associated with specific task requirements. For example, a recent study has shown that the effect of stimulus emotionality on pupil dilation is reduced in older adults (Hämmerer et al., 2017). Thus, although these arousal systems appear unchanged in the early stages of aging studied here, aging-related changes associated with particular cognitive contexts might be uncovered in future studies. Moreover, additional preliminary analyses of our own pupil data suggested that older adults show different dynamics of the pupillary signal revealed by significant group differences in the amplitude of the time derivative of the pupillary responses and latency of the peak response. These observations would need to be further explored in future studies.

Pupil dilation is commonly observed in cued RT tasks during the preparatory period between cue and target (Jainta, 2011; Jennings et al., 1998; Moresi et al., 2008, 2011; Tona et al., 2016), suggesting that preparatory processes involve an increase in arousal levels. In our study, by comparing the response during the passive perception of the cue stimulus with the cue-only trials and cue-target trials of the active tasks, we were able to describe the full dynamics of pupil dilation responses associated with preparatory processes. We observed a biphasic response to the cue in cue-only trials, where the early peak was related to the auditory processing of the cue stimulus and did not differ between passive and active tasks. The properties of this pupillary response are similar to the early nondiscriminative LC response detected in electrophysiological recordings in monkeys in target and nontarget trials and closely linked to sensory stimuli (Rajkowski et al., 2004). The latter peak was specific for the active tasks and therefore must represent preparatory processes related to the orientation of attention in anticipation and preparation to process and respond to incoming stimuli. Preparatory processes were associated with a large pupillary response with very similar dynamics to the pupillary response observed in cue-target trials, revealing that pupil size increased during the trial while participants were expecting the target to appear and preparing to respond. The association of pupillary responses with motoric preparation is in line with observations from previous studies. In go/no-go tasks with differing go probabilities, increasing the go probability increases the pupil dilation in no-go trials (van der Molen et al., 1989). With high go probability, participants prepare for response in all trials even before the go/no-go stimulus is displayed. With low go probability, participants wait for the stimulus before preparing for action, thereby showing minimal response preparation in no-go trials. Pupil dilation appears to reflect these differences in motor preparation. Given the high go probability employed in our study, it is likely that participants prepared for action in all trials, thereby showing similar pupil dilation responses in cue-only and cue-target trials, and go and no-go trials. Notably, the fact that we do not observe significant differences when comparing go and no-go trials suggests that the amplitude of the pupil dilation reflects motor preparation and not the motor response, which was absent in the no-go trials. Furthermore, our findings confirm previous findings: in go/no-go tasks with high go probability, the amplitude of the pupil dilation in go and no-go trials is very similar (Richer et al., 1983; van der Molen et al., 1989).

Preparation in the go/no-go task elicited larger pupil dilations than that in the simple RT task indicating that preparing for a choice task was associated with higher engagement of arousal systems in comparison with preparing for a detection task. The lack of group differences in these pupillary preparatory processes suggested that engagement of the arousal systems during preparation was similar in young and older adults. This finding contrasted with our ERP results that revealed enhanced cortical responses during the preparatory cue-target period in the older adults and suggested a dissociation between preparatory cortical frontocentral activity and preparatory modulation of arousal as measured by pupillary responses. Furthermore, we observed that while, in young adults, the amplitude of the frontocentral preparatory ERPs was modulated by task demands (higher in the go/no-go task than in the simple RT task), in older adults, this modulation was reduced. These observations suggest that older adults recruit more neural resources in the preparation to process and respond to the incoming stimuli but fail to modulate this recruitment according to task demands as the young participants do. Hyperactivation of brain areas by older adults during task performance has been postulated as being a compensatory mechanism by which the recruitment of additional regions supports the cognitive functions compromised by age-related deficits (Reuter-Lorenz and Park, 2014). The way these additional brain regions support cognition is still unclear and most likely depends on the task and cognitive domain. One hypothesis is that these areas are domain-general and that assist with task performance by enhancing working memory and executive control. In a meta-analysis of neuroimaging studies comparing young and older adults, it was found that, across cognitive domains, older adults recruit to a greater extent left and right dorsolateral prefrontal cortex, left rostrolateral prefrontal cortex, and regions within the right precentral sulcus (Spreng et al., 2010). Higher prefrontal engagement in older adults might reflect a higher level of top-down (proactive) control (Di Russo et al., 2017). However, the cognitive strategy used might change according to the task demands. Indeed, in more demanding tasks requiring inhibition or active maintenance of and shifts in task rules, older adults change from a more proactive to a more reactive strategy (Di Russo et al., 2017; Hämmerer et al., 2014). In these cases, older individuals might present lower prefrontal activation than young adults.

Our results suggest a dissociation between ERPs and pupillary signals. Previous studies also suggested that these different aspects of neural preparation are independent and contribute separately to task performance (Jennings and Van Der Molen, 2005), in line with the lack of correlation between these variables observed before (Jennings et al., 1998). However, the dissociation between activation of the frontal cortex, in particular, and activation of brainstem arousal systems is surprising given the reciprocal connections that exist between them (Gompf et al., 2010). It is possible, however, that the relation between these 2 systems is reflected in other measures of cortical activity. In fact, the amplitude of midline theta oscillations has been shown to correlate with pupil dilation in 2 recent studies (Dippel et al., 2017; Lin et al., 2018). Theta oscillations are observed during and are linked to motor preparation (Tomassini et al., 2017). Further studies are needed to resolve if the amplitude of pupillary responses and premotor theta oscillations are related in cued RT tasks.

In the time window around button press, parietal and occipital electrodes presented a positive potential, which amplitude was reduced in the older group. This parietal ERP presented higher amplitude in the go/no-go task than in the simple RT task; however, this task modulation was reduced in the older group, suggesting, once more, reduced modulation of the recruitment of neural resources according to task demands. This parietal response resembled the P300 potential, a positive deflection observed over the parietal scalp in response to task-relevant stimuli, considered to be a correlate of allocation of attention during stimulus categorization (Kok, 2001; Polich, 2007). A meta-analysis of P300 studies confirmed that the amplitude of this electrophysiological response decreases with aging (Dinteren et al., 2014). Notably, P300 share a lot in common with LC phasic activity (Nieuwenhuis et al., 2011). This fact has led to the suggestion that P300 reflects the noradrenergic input from the LC into the cortex. However, although in previous studies, pretarget (baseline) pupil diameter has been found to relate to the amplitude of P300, the amplitude of pupil dilation did not correlate with the P300 amplitude (Hong et al., 2014; Kamp and Donchin, 2015; Murphy et al., 2011). Therefore, although these variables are related, the mechanism behind this association is still a matter for debate. Moreover, in our study, we observed reduced amplitude of P300 in the older group and no differences in the amplitude of pupillary responses suggesting that the age-related reduction in P300 amplitude is not a consequence of reduced task-related activation of the LC.

The amplitude reduction in the ERPs observed in older adults at the time of the button press extended beyond the motor response. In these types of tasks, neural activity that occurs after the motor response is probably associated with processes involved in response monitoring, performance evaluation, update of task-related representations, and adjustments in task performance. Previous studies have shown that performance monitoring is less efficient in older adults. Error awareness in older adults is reduced, slower, and more variable, and it has been associated with reduced neural activation during the post–error period (Harty et al., 2017; Niessen et al., 2017). Given the small number of errors participants committed in our study, we were not able to investigate neural activity associated with error processing. Nevertheless, our findings suggest reduced activity levels in older adults also after correct responses that might contribute to the decrement in performance monitoring associated with aging (Eppinger et al., 2011).

In summary, in comparison with young adults, older adults recruited more neural resources during preparatory processes reflected in enhanced frontocentral ERPs yet presented reduced parietal activation at the time of the motor response. These observations suggest that, in cued RT tasks, the older brain employs larger neural resources at the beginning of the trial but reduce neural engagement at the end of the trial. Concomitantly, the amplitude of the pupillary responses associated with the preparation to process and respond to incoming stimuli as well as pupillary responses during target processing, response selection, and button press did not show an aging effect, suggesting a dissociation between cortical processing and task-related arousal modulation. Given the well-established link between pupil size and activity in the noradrenergic nucleus, LC, our findings suggest that, in the early stages of aging investigated here, the level of recruitment of this brainstem nucleus is not significantly affected during performance of cued RT tasks even in the presence of age-related differences in behavioral performance and cortical processing.

## Disclosure statement

All authors report no actual or potential conflicts of interest.

## Acknowledgements

The authors would like to thank Mr. Marco Simões and Prof. Cyril Pernet for their assistance in the implementation of the analysis with the LIMO EEG toolbox. Moreover, the authors would like to thank all the participants that kindly agreed to participate in this study and the Coimbra Universities of Third Age, Instituto Humanus, Associação Apojovi Universidade Aposenior, and Universidade do Tempo Livre (ANAI), for their collaboration in participant recruitment. This work was funded by Fundação para a Ciência e a Tecnologia (Grants: SFRH/BPD/102188/2014 and UID/NEU/04539/2013-COMPETE, POCI-01-0145-FEDER-00 7440, PAC–MEDPERSYST, POCI-01-0145-FEDER-30852, POCI-01-0145-

FEDER-016428, BIGDATIMAGE, CENTRO-01-0145-FEDER-000016, financed by Centro 2020 FEDER, COMPETE).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.neurobiolaging.2018.09.028.

## References

Alexander, J.K., Hillier, A., Smith, R.M., Tivarus, M.E., Beversdorf, D.Q., 2007. Beta-adrenergic modulation of cognitive flexibility during stress. (Cognitive flexibility). J. Cogn. Neurosci. 19, 468–478.
Aston-Jones, G., Cohen, J.D., 2005. An integrative theory of locus coeruleus-norepinephrine function: adaptive gain and optimal performance. Annu. Rev. Neurosci. 28, 403–450.
Aston-Jones, G., Waterhouse, B., 2016. Locus coeruleus: from global projection system to adaptive regulation of behavior. Brain Res. 1645, 75–78.
Bellec, P., Lavoie-Courchesne, S., Dickinson, P., Lerch, J.P., Zijdenbos, A.P., Evans, A.C., 2012. The pipeline system for Octave and Matlab (PSOM): a lightweight scripting framework and execution engine for scientific workflows. Front. Neuroinform. 6, 1–18.
Berchicci, M., Lucci, G., Pesce, C., Spinelli, D., Di Russo, F., 2012. Prefrontal hyperactivity in older people during motor planning. Neuroimage 62, 1750–1760.
Brainard, D.H., 1997. The psychophysics tollbox. Spat. Vis. 10, 433–436.
Brooks, J.C.W., Faull, O.K., Pattinson, K.T.S., Jenkinson, M., 2013. Physiological noise in brainstem fMRI. Front. Hum. Neurosci. 7, 1–13.
Brown, S.B., Slagter, H.A., Van Noorden, M.S., Giltay, E.J., Van Der Wee, N.J., Nieuwenhuis, S., 2016. Effects of clonidine and scopolamine on multiple target detection in rapid serial visual presentation. Psychopharmacology (Berl) 233, 341–350.
Campbell, H., Tivarus, M., Hillier, A., Beversdorf, D., 2008. Increased task difficulty results in greater impact of noradrenergic modulation of cognitive flexibility. Pharmacol. Biochem. Behav. 88, 222–229.
Chandler, D.J., Gao, W.-J., Waterhouse, B.D., 2014. Heterogeneous organization of the locus coeruleus projections to prefrontal and motor cortices. Proc. Natl. Acad. Sci. U S A 111, 6816–6821.
Clayton, E.C., 2004. Phasic activation of monkey locus ceruleus neurons by simple decisions in a forced-choice task. J. Neurosci. 24, 9914–9920.
Clewett, D.V., Lee, T.H., Greening, S., Ponzio, A., Margalit, E., Mather, M., 2015. Neuromelanin marks the spot: identifying a locus coeruleus biomarker of cognitive reserve in healthy aging. Neurobiol. Aging 37, 117–126.
Clewett, D., Huang, R., Velasco, R., Lee, T.-H., Mather, M., 2018. Locus coeruleus activity strengthens prioritized memories under arousal. J. Neurosci. 38, 1558–1574.
Coull, J.T., Nobre, A.C., Frith, C.D., 2001. The noradrenergic α2 agonist clonidine modulates behavioural and neuroanatomical correlates of human attentional orienting and alerting. Cereb. Cortex. 11, 73–84.
de Gee, J.W., Knapen, T., Donner, T.H., 2014. Decision-related pupil dilation reflects upcoming choice and individual bias. Proc. Natl. Acad. Sci. U S A 111, E618–E625.
de Gee, J.W., Colizoli, O., Kloosterman, N.A., Knapen, T., Nieuwenhuis, S., Donner, T.H., 2017. Dynamic modulation of decision biases by brainstem arousal systems. Elife 6, e23232.
Der, G., Deary, I.J., 2009. Age and sex differences in reaction time in adulthood: results from the United Kingdom health and lifestyle survey": correction. Psychol. Aging 24, 229.
Di Russo, F., Berchicci, M., Bozzacchi, C., Perri, R.L., Pitzalis, S., Spinelli, D., 2017. Beyond the "Bereitschaftspotential": action preparation behind cognitive functions. Neurosci. Biobehav. Rev. 78, 57–81.
Dinteren, R., Arns, M., Jongsma, M.L., Kessels, R.P., 2014. P300 development across the lifespan: a systematic review and meta-analysis. PLoS One 9, e87347.
Dippel, G., Mückschel, M., Ziemssen, T., Beste, C., 2017. Demands on response inhibition processes determine modulations of theta band activity in superior frontal areas and correlations with pupillometry – implications for the norepinephrine system during inhibitory control. Neuroimage 157, 575–585.
Duque, J., Petitjean, C., Swinnen, S.P., 2016. Effect of aging on motor inhibition during action preparation under sensory conflict. Front. Aging Neurosci. 8, 1–14.
Eldar, E., Cohen, J.D., Niv, Y., 2013. The effects of neural gain on attention and learning. Nat. Neurosci. 16, 1146–1153.
Eppinger, B., Hämmerer, D., Li, S.C., 2011. Neuromodulation of reward-based learning and decision making in human aging. Ann. N. Y. Acad. Sci. 1235, 1–17.
Featherstone, J.A., Veith, R.C., Flatness, D., Murburg, M.M., Villacres, E.C., Halter, J.B., 1987. Age and alpha-2 adreneric regulation of plasma norepinephrine linetics in humans. J. Gerontol. 42, 271–276.
Fernandez-Ruiz, J., Peltsch, A., Alahyane, N., Brien, D.C., Coe, B.C., Garcia, A., Munoz, D.P., 2018. Age related prefrontal compensatory mechanisms for inhibitory control in the antisaccade task. Neuroimage 165, 92–101.
Freitas, S., Simões, M.R., Alves, L., Santana, I., 2011. Montreal cognitive assessment (MoCA): normative study for the Portuguese population. J. Clin. Exp. Neuropsychol. 33, 989–996.
Gompf, H.S., Mathai, C., Fuller, P.M., Wood, D.A., Pedersen, N.P., Saper, C.B., Lu, J., 2010. Locus ceruleus and anterior cingulate cortex sustain wakefulness in a novel environment. J. Neurosci. 30, 14543–14551.
Granholm, E., Morris, S., Asarnow, R.F., Chock, D., Jeste, D.V., 2000. Accelerated age-related decline in processing resources in schizophrenia: evidence from pupillary responses recorded during the span of apprehension task. J. Int. Neuropsychol. Soc. 6, 30–43.
Grefkes, C., Wang, L.E., Eickhoff, S.B., Fink, G.R., 2010. Noradrenergic modulation of cortical networks engaged in visuomotor processing. Cereb. Cortex. 20, 783–797.
Halliday, R., Callaway, E., Lannon, R., 1989. The effects of clonidine and yohimbine on human information processing. Psychopharmacology (Berl) 99, 563–566.
Hämmerer, D., Callaghan, M.F., Hopkins, A., Betts, M., Kosciessa, J.Q., Cardenas-Blanco, A., Kanowski, M., Weiskopf, N., Dayan, P., Dolan, R.J., Düzel, E., 2018. Locus Coeruleus integrity in old age is selectively related to memories linked with salient negative events. Proc. Natl. Acad. Sci. U S A 115, 2228–2233.
Hämmerer, D., Müller, V., Li, S.C., 2014. Performance monitoring across the lifespan: still maturing post-conflict regulation in children and declining task-set monitoring in older adults. Neurosci. Biobehav. Rev. 46, 105–123.
Hämmerer, D., Hopkins, A., Betts, M.J., Maaß, A., Dolan, R.J., Düzel, E., 2017. Emotional arousal and recognition memory are differentially reflected in pupil diameter responses during emotional memory for negative events in younger and older adults. Neurobiol. Aging 58, 129–139.
Harty, S., Murphy, P.R., Robertson, I.H., O'Connell, R.G., 2017. Parsing the neural signatures of reduced error detection in older age. Neuroimage 161, 43–55.
Hong, L., Walz, J.M., Sajda, P., 2014. Your eyes give you away: prestimulus changes in pupil diameter correlate with poststimulus task-related EEG dynamics. PLoS One 9, e91321.
Hurlemann, R., 2005. Noradrenergic modulation of emotion-induced forgetting and remembering. J. Neurosci. 25, 6343–6349.
Ishida, Y., Shirokawa, T., Miyaishi, O., Komatsu, Y., Isobe, K., 2000. Age-dependent changes in projections from locus coeruleus to hippocampus dentate gyrus and frontal cortex. Eur. J. Neurosci. 12, 1263–1270.
Jainta, S., 2011. The pupil reflects motor preparation for saccades – even before the eye starts to move. Front. Hum. Neurosci. 5, 1–10.
Jennings, J.R., Van Der Molen, M.W., 2005. Preparation for speeded action as a psychophysiological concept. Psychol. Bull. 131, 434–459.
Jennings, J.R., van der Molen, M.W., Steinhauer, S.R., 1998. Preparing the heart, eye, and brain: foreperiod length effects in a nonaging paradigm. Psychophysiology 35, 90–98.
Joshi, S., Li, Y., Kalwani, R.M., Gold, J.I., 2016. Relationships between pupil diameter and neuronal activity in the locus coeruleus, colliculi, and cingulate cortex. Neuron 89, 221–234.
Kamp, S.M., Donchin, E., 2015. ERP and pupil responses to deviance in an oddball paradigm. Psychophysiology 52, 460–471.
Kaplon, R.E., Walker, A.E., Seals, D.R., 2011. Plasma norepinephrine is an independent predictor of vascular endothelial function with aging in healthy women. J. Appl. Physiol. 111, 1416–1421.
Kok, A., 2001. On the utility of P3 amplitude as a measure of processing capacity. Psychophysiology 38, 557–577.
Kubis, N., Faucheux, B.A., Ransmayr, G., Damier, P., Duyckaerts, C., Henin, D., Forette, B., Le Charpentier, Y., Hauw, J.-J., Agid, Y., Hirsch, E.C., 2000. Preservation of midbrain catecholaminergic neurons in very old human subjects. Brain 123, 366–373.
Lin, H., Saunders, B., Hutcherson, C.A., Inzlicht, M., 2018. Midfrontal theta and pupil dilation parametrically track subjective conflict (but also surprise) during intertemporal choice. Neuroimage 172, 838–852.
Manaye, K.F., McIntire, D.D., Mann, D.M.A., German, D.C., 1995. Locus coeruleus cell loss in the aging human brain: a non-random process. J. Comp. Neurol. 358, 79–87.
Maris, E., Oostenveld, R., 2007. Nonparametric statistical testing of EEG- and MEG-data. J. Neurosci. Methods 164, 177–190.
Mather, M., Harley, C.W., 2016. The locus coeruleus: essential for maintaining cognitive function and the aging brain. Trends Cogn. Sci. 20, 214–226.
Moresi, S., Adam, J.J., Rijcken, J., Van Gerven, P.W.M., Kuipers, H., Jolles, J., 2008. Pupil dilation in response preparation. Int. J. Psychophysiol. 67, 124–130.
Moresi, S., Adam, J.J., Rijcken, J., Kuipers, H., Severens, M., Van Gerven, P.W.M., 2011. Response preparation with adjacent versus overlapped hands: a pupillometric study. Int. J. Psychophysiol. 79, 280–286.
Mückschel, M., Chmielewski, W., Ziemssen, T., Beste, C., 2017. The norepinephrine system shows information-content specific properties during cognitive control – evidence from EEG and pupillary responses. Neuroimage 149, 44–52.
Murphy, P.R., Robertson, I.H., Balsters, J.H., O'connell, R.G., 2011. Pupillometry and P3 index the locus coeruleus-noradrenergic arousal function in humans. Psychophysiology 48, 1532–1543.
Murphy, P.R., O'Connell, R.G., O'Sullivan, M., Robertson, I.H., Balsters, J.H., 2014. Pupil diameter covaries with BOLD activity in human locus coeruleus. Hum. Brain Mapp. 35, 4140–4154.
Niccoli, T., Partridge, L., 2012. Ageing as a risk factor for disease. Curr. Biol. 22, R741–R752.
Niessen, E., Fink, G.R., Hoffmann, H.E.M., Weiss, P.H., Stahl, J., 2017. Error detection across the adult lifespan: electrophysiological evidence for age-related deficits. Neuroimage 152, 517–529.

Nieuwenhuis, S., van Nieuwpoort, I.C., Veltman, D.J., Drent, M.L., 2007. Effects of the noradrenergic agonist clonidine on temporal and spatial attention. Psychopharmacology (Berl) 193, 261–269.
Nieuwenhuis, S., De Geus, E.J., Aston-Jones, G., 2011. The anatomical and functional relationship between the P3 and autonomic components of the orienting response. Psychophysiology 48, 162–175.
Pernet, C.R., Chauveau, N., Gaspar, C., Rousselet, G.A., 2011. LIMO EEG: a toolbox for hierarchical linear modeling of electroencephalographic data. Comput. Intell. Neurosci. 2011, 831409.
Pernet, C.R., Latinus, M., Nichols, T.E., Rousselet, G.A., 2015. Cluster-based computational methods for mass univariate analyses of event-related brain potentials/fields: a simulation study. J. Neurosci. Methods 250, 85–93.
Plewnia, C., Hoppe, J., Hiemke, C., Bartels, M., Cohen, L.G., Gerloff, C., 2002. Enhancement of human cortico-motoneuronal excitability by the selective norepinephrine reuptake inhibitor reboxetine. Neurosci. Lett. 330, 231–234.
Polich, J., 2007. Updating P300: an integrative theory of P3a and P3b. Clin. Neurophysiol. 118, 2128–2148.
Porter, G., Tales, A., Troscianko, T., Wilcock, G., Haworth, J., Leonards, U., 2010. New insights into feature and conjunction search: I. Evidence from pupil size, eye movements and ageing. Cortex 46, 621–636.
Rajkowski, J., Majczynski, H., Clayton, E., Aston-Jones, G., 2004. Activation of monkey locus coeruleus neurons varies with difficulty and performance in a target detection task. J. Neurophysiol. 92, 361–371.
Reimer, J., Mcginley, M.J., Liu, Y., Rodenkirch, C., Wang, Q., Mccormick, D.A., Tolias, A.S., 2016. Pupil fluctuations track rapid changes in adrenergic and cholinergic activity in cortex. Nat Commun. 7, 13289.
Reuter-Lorenz, P.A., Cappell, K.A., 2008. Neurocognitive aging and the compensation hypothesis. Curr. Dir. Psychol. Sci. 17, 177–182.
Reuter-Lorenz, P.A., Park, D.C., 2014. How does it STAC up? Revisiting the scaffolding theory of aging and cognition. Neuropsychol. Rev. 24, 355–370.
Richer, F., Silverman, C., Beatty, J., 1983. Response selection and initiation in speeded Reactions : a pupillometric analysis. J. Exp. Psychol. Hum. Percept. Perform. 9, 360–370.
Robertson, I.H., 2013. A noradrenergic theory of cognitive reserve: implications for Alzheimer's disease. Neurobiol. Aging 34, 298–308.
Samuels, E.R., Szabadi, E., 2008. Functional neuroanatomy of the noradrenergic locus coeruleus: its roles in the regulation of arousal and autonomic function part II: physiological and pharmacological manipulations and pathological alterations of locus coeruleus activity in humans. Curr. Neuropharmacol. 6, 254–285.
Shibata, E., Sasaki, M., Tohyama, K., Kanbara, Y., Otsuka, K., Ehara, S., Sakai, A., 2006. Age-related changes in locus ceruleus on neuromelanin magnetic resonance imaging at 3 Tesla. Magn. Reson. Med. Sci. 5, 197–200.
Siegle, G.J., Ichikawa, N., Steinhauer, S., 2008. Blink before and after you think: blinks occur prior to and following cognitive load indexed by pupillary responses. Psychophysiology 45, 679–687.
Siegle, G.J., Steinhauer, S.R., Stenger, V.A., Konecky, R., Carter, C.S., 2003. Use of concurrent pupil dilation assessment to inform interpretation and analysis of fMRI data. Neuroimage 20, 114–124.
Spreng, R.N., Wojtowicz, M., Grady, C.L., 2010. Reliable differences in brain activity between young and old adults: a quantitative meta-analysis across multiple cognitive domains. Neurosci. Biobehav. Rev. 34, 1178–1194.
Sterr, A., Dean, P., 2007. Neural correlates of movement preparation in healthy ageing. Eur. J. Neurosci. 27, 254–260.
Swann, A.C., Birnbaum, D., Jagar, A.A., Dougherty, D.M., Moeller, F.G., 2005. Acute yohimbine increases laboratory-measured impulsivity in normal subjects. Biol. Psychiatry 57, 1209–1211.
Swann, A.C., Lijffijt, M., Lane, S.D., Cox, B., Steinberg, J.L., Moeller, F.G., 2013. Norepinephrine and impulsivity: effects of acute yohimbine. Psychopharmacology (Berl) 229, 83–94.
Tomassini, A., Ambrogioni, L., Medendorp, W.P., Maris, E., 2017. Theta oscillations locked to intended actions rhythmically modulate perception. Elife 6, e25618.
Tona, K.-D., Murphy, P.R., Brown, S.B., Nieuwenhuis, S., 2016. The accessory stimulus effect is mediated by phasic arousal: a pupillometry study. Psychophysiology 53, 1108–1113.
van der Molen, M.W., Boomsma, D.I., Jennings, J.R., Nieuwboer, R.T., 1989. Does the heart know what the eye sees? A cardiac/pupillometric analysis of motor preparation and response execution. Psychophysiology 26, 70–80.
Van Gerven, P.W.M., Paas, F., Van Merrienboer, J.J.G., Schmidt, H.G., 2004. Memory load and the cognitive pupillary response in aging. Psychophysiology 41, 167–174.
Wang, L.Y., Murphy, R.R., Hanscom, B., Li, G., Millard, S.P., Petrie, E.C., Galasko, D.R., Sikkema, C., Raskind, M.A., Wilkinson, C.W., Peskind, E.R., 2013. Cerebrospinal fluid norepinephrine and cognition in subjects across the adult age span. Neurobiol. Aging 34, 2287–2292.
Wild-Wall, N., Hohnsbein, J., Falkenstein, M., 2007. Effects of ageing on cognitive task preparation as reflected by event-related potentials. Clin. Neurophysiol. 118, 558–569.
Wilson, R.S., Nag, S., Boyle, P.A., Hizel, L.P., Yu, L., Buchman, A.S., Schneider, J.A., Bennett, D.A., 2013. Neural reserve, neuronal density in the locus ceruleus, and cognitive decline. Neurology 80, 1202–1208.
Woods, D.L., Wyma, J.M., Yund, E.W., Herron, T.J., Reed, B., 2015a. Factors influencing the latency of simple reaction time. Front. Hum. Neurosci. 9, 131.
Woods, D.L., Wyma, J.M., Yund, E.W., Herron, T.J., Reed, B., 2015b. Age-related slowing of response selection and production in a visual choice reaction time task. Front. Hum. Neurosci. 9, 193.
Wyss-Coray, T., 2016. Ageing, neurodegeneration and brain rejuvenation. Nature 539, 180–186.
Yordanova, J., Kolev, V., Hohnsbein, J., Falkenstein, M., 2004. Sensorimotor slowing with ageing is mediated by a functional dysregulation of motor-generation processes: evidence from high-resolution event-related potentials. Brain 127, 351–362.