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Neural Dynamics of Multiple Object Processing in Mild Cognitive Impairment and Alzheimer's Disease: Future Early Diagnostic Biomarkers?

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Abstract. The aim of this study was to investigate the behavioral and electrophysiological dynamics of multiple object processing (MOP) in mild cognitive impairment (MCI) and Alzheimer's disease (AD), and to test whether its neural signatures may represent reliable diagnostic biomarkers. Behavioral performance and event-related potentials [N2pc and contralateral delay activity (CDA)] were measured in AD, MCI, and healthy controls during a MOP task, which consisted in enumerating a variable number of targets presented among distractors. AD patients showed an overall decline in accuracy for both small and large target quantities, whereas in MCI patients, only enumeration of large quantities was impaired. N2pc, a neural marker of attentive individuation, was spared in both AD and MCI patients. In contrast, CDA, which indexes visual short term memory abilities, was altered in both groups of patients, with a non-linear pattern of amplitude modulation along the continuum of the disease: a reduction in AD and an increase in MCI. These results indicate that AD pathology shows a progressive decline in MOP, which is associated to the decay of visual short-term memory mechanisms. Crucially, CDA may be considered as a useful neural signature both to distinguish between healthy and pathological aging and to characterize the different stages along the AD continuum, possibly becoming a reliable candidate for an early diagnostic biomarker of AD pathology.

Keywords: Alzheimer's disease, attention, biomarkers, electroencephalography, event-related potentials, mild cognitive impairment, short-term memory

INTRODUCTION

Alzheimer's disease (AD) is the most frequent cause of age-related dementia, accounting for about 60% of cases, and its incidence is expecting to raise more and more, as around 80 million of dementia cases have been foreseeable by 2040 [1]. Current evidence considers AD as a portion of a biological and clinical continuum with amyloid plaques deposition starting even 10–30 years before the onset of the first clinical symptoms [2]. This continuum may be divided in three main stages [3], ranging from the initial preclinical phase (healthy individuals with no cognitive symptoms who present AD pathological changes) to the final dementia phase with the well-known multidomain cognitive and functional impairment. Mild cognitive impairment (MCI) syndrome occurs between these stages. MCI is defined as

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the prodromal phase of AD [4], and is characterized by cognitively impaired individuals who do not meet the criteria for dementia and show an overall preservation of everyday activities [5]. Patients with MCI are considered at high-risk for the development of AD, with a conversion rate of about 10–15% within one year [6]. Identification of neural markers that can distinguish between healthy elderly and patients at early stages of the disease progression is a great challenge and is crucial to perform an early diagnosis and to intervene early in the disease progression.

Behavioral and event-related potentials (ERPs) paradigms coming from cognitive neuroscience have been proven to be particularly useful in the attempt to characterize and distinguish healthy elderly from early-stage AD patients and even to predict which prodromal patients will convert in AD [7, 8].

In the present study, we will focus on a paradigm that has been used to investigate attention and working memory by means of tasks requiring participants to respond to multiple objects. Processing multiple objects concurrently is a fundamental ability to have a coherent perception of the environment and to successfully interact with it: in every moment of our daily life we are required to track a multitude of objects that are usually surrounded by other distractor objects, as in the case of driving a car in a congested highway. The investigation of the behavioral and neural dynamics of multiple objects processing (MOP) may provide pivotal hints in the study of pathological aging, particularly in those diseases characterized by memory and attentional impairment, such as AD [9]. Thus, MOP paradigms may be crucial for the characterization of the pathophysiological mechanisms underlying AD and the identification of its neural markers for an early diagnosis. Recent brain imaging studies reported that MOP mainly activates temporo-parietal areas [10–12], that are among the key regions early affected by AD and which suffer from the earliest deposition of amyloid-β, even before cognitive symptoms appear [13]. Indeed, FDG-PET studies in patients with AD showed a reduced metabolism in the temporo-parietal cortex, posterior cingulate, and precuneus [14].

To investigate MOP, several studies adopted visual enumeration tasks in which participants are requested to count a variable number of target stimuli presented among distractors. A peculiar feature of visual enumeration is the so-called "subitizing phenomenon" [15], which is represented by the different performance in enumerating a small or a larger amount of items. When participants have to enumerate a relatively small number of items (usually up to 3-4), they are fast and accurate ("subitizing"), while when the number of objects to be enumerated is larger they are more error prone and their reaction times deeply increase (a process usually referred to as "counting"). MOP involves two main mechanisms: an early attentive individuation mechanism, which provides a coarse representation of the items simultaneously, and a later mechanism, which encodes the individuated items in greater detail and relies on visual short-term memory (VSTM). The involvement of both attention and VSTM mechanisms in the simultaneous processing of multiple objects has been further substantiated by some electroencephalographic studies in early adulthood (for a review, see [16]) and in healthy aging [17]. These studies focused on two ERP components, the N2pc [18, 19] and contralateral delay activity (CDA) [20], that have been associated to attentive individuation and VSTM processes, respectively. The N2pc was originally measured in visual search tasks where a target is presented among several distractors, and reflects the process of target selective individuation. The CDA has typically been measured in memory tasks with multiple elements, and has been interpreted as the signature of the active maintenance of a limited set of items in a temporary buffer. These components are modulated by several factors, including stimulus-driven grouping [21–23] as well as task instructions [24, 25]. Importantly, in enumeration tasks where multiple targets are presented among distractors, the N2pc amplitude increases as a function of target numerosity, reaching a limit at around three targets [26]. In these tasks, the CDA likely indexes the encoding and maintaining of multiple objects in VSTM for quantity-to-symbol mapping. As for the N2pc, its amplitude increases as a function of the number of objects that have to be maintained in memory storage, reaching an asymptotic limit at around three objects [20]. Enumeration abilities have been sparingly investigated in AD patients, while to our knowledge, no studies have adopted this paradigm in preclinical or prodromal population such as MCI patients. The few behavioral studies on enumeration in AD patients have highlighted a reduction of subitizing span (i.e., the number of targets that are processed simultaneously) in AD patients in comparison to healthy elderly controls, and a significant correlation between the overall enumeration performance and the severity of the disease [27–29].

In the present study, we recorded electroencephalogram (EEG) activity while mild AD patients, MCI

patients, and healthy elderly controls (HC) performed a multiple objects enumeration task. In terms of EEG responses, we focused on the aforementioned electrophysiological components associated to attentive individuation and VSTM-related processes, N2pc and CDA. The aim of this study was two-fold: to investigate the behavioral and electrophysiological dynamics of MOP along the continuum of AD severity (from healthy aging to mild AD); and to test whether N2pc and/or CDA may characterize the progression of the attention and memory impairment, thus possibly becoming reliable candidates for diagnostic neural markers of AD pathology.

MATERIALS AND METHODS

Participants

Twenty mild AD patients, 16 amnestic MCI patients (both single and multi-domain), and 20 HC, aged 60 to 85 years, were recruited (Table 1). Inclusion criteria for AD patients were a diagnosis of probable AD according to [30], Mini-Mental State Examination (MMSE) score greater or equal to 20 and a Clinical Dementia Rating scale (CDR) score less than or equal to 2. All patients had been on a stable dose of cholinesterase inhibitors (donepezil or rivastigmine) for at least 3 months prior to participation in the study. Inclusion criteria for MCI patients [6] were a MMSE score greater or equal to 24 and a CDR score equal to 0.5. Inclusion criteria for HC were the absence of previous history of neurological or psychiatric problems, an MMSE score between 24 and 30 and a CDR score of 0. Exclusion criteria were the presence of medical, neurological or psychiatric disorders that might interfere with the study and a Hachinski Ischemia score greater than 4. All participants underwent a detailed neuropsychological assessment (Table 1).

All participants gave their written informed consent prior to the beginning of the experiment. All procedures were approved by the Ethics Committee of the IRCCS San Giovanni di Dio Fatebenefratelli Scientific Institute (Brescia, Italy) and were performed according to the Declaration of Helsinki for research involving human subjects.

Stimuli and procedure

To investigate MOP, we adopted a visual enumeration task where participants were requested to count a variable number (ranging from 1 to 6) of targets (green dots) presented among distractors (red dots). Stimuli and procedures are comprehensively described in [17, 31, 32]. Participants were seated in a dimly lit room in front of a 17″ Dell monitor at a viewing distance of approximately 80 cm. Each trial began with the fixation dot displayed for a random interval (ranging from 2460 to 2540 ms). The stimulus array was then displayed for 400 ms. After a blank frame lasting 500 ms, the response screen was displayed until the participant's response. In light of the acknowledged inability of AD patients to covertly orient attention and, thus, to inhibit saccades toward stimuli when they appear [33, 34], all participants were not explicitly requested to maintain their gaze on the central fixation dot during stimulus presentation, but instead to have their eyes on the fixation dot when each trial started. Otherwise, we would not have a comparable condition among the three groups. Participants were required to count the number of targets (from 1 to 6) and verbally report the response. A total of 600 trials (10 blocks, 60 trials each) was delivered in the experimental session, preceded by one practice block (18 trials). The stimuli were generated, and responses were recorded using E-Prime 2 software (Psychology Software Tools, Pittsburgh, PA).

EEG recording and processing

EEG was continuously recorded from an ActiCAP cap with 27 active Ag/AgCl electrodes (Brain Products, GmbH, Munich, Germany) placed according to the 10–20 International System and comprising: Fp1, Fp2, F7, F3, Fz, F4, F8, FCz, T7, C3, Cz, C4, T8, CP5, CP6, P7, P3, Pz, P4, P8, PO7, PO9, PO8, PO10, O1, Oz, O2. The signal was referenced online to the right mastoid, and then re-referenced offline to the average of the left and right mastoids. The ground electrode was placed over AFz. Horizontal and vertical eye movements were detected respectively with electrodes placed at the left and right canthi and above and below the right eye. The EEG was recorded at 1000 Hz sampling rate with a time constant of 10 s as a low cut-off filter and a high cut-off of 250 Hz. The EEG signal was processed and analyzed offline using Brain Vision Analyzer 2 (Brain Products, GmbH, Munich, Germany). Continuous data were filtered off-line with a 40 Hz high cut-off filter. Brain components corresponding to ocular (blinks and saccades) and related artifacts were identified and discarded using the ICA ocular correction

Table 1
Demographic and age- and education-adjusted neuropsychological results for the three groups of participants (AD, MCI, and HC) reported as mean (±SEM)

	AD	MCI	HC	F	*p*	AD versus HC	MCI versus HC	AD versus MCI
Age (years)	76.30 (1.52)	75.00 (1.56)	69.40 (0.95)	4.06	**	***	*	0.88
Education (years)	7.80 (0.63)	8.19 (0.77)	10.65 (0.91)	8.58	*	*	0.10	0.98
MMSE	22.07 (0.32)	26.16 (0.45)	27.78 (0.44)	57.11	***	***	*	***
RCPM 47	26.30 (1.38)	30.84 (0.91)	32.08 (0.79)	8.34	***	***	0.821	*
RAVLT-immediate recall	28.00 (1.44)	37.86 (2.20)	45.81 (1.45)	30.66	***	***	**	***
RAVLT-delayed recall	2.99 (0.40)	7.00 (0.81)	10.22 (0.45)	47.89	***	***	***	***
Episodic memory	2.43 (0.45)	7.63 (1.21)	14.63 (0.76)	61.74	***	***	***	***
ROCF-copy	27.26 (1.99)	33.41 (1.23)	35.64 (0.37)	10.95	***	***	0.568	**
ROCF-recall	7.69 (0.82)	12.68 (1.87)	18.71 (1.07)	19.93	***	***	**	*
Digit Span	5.50 (0.21)	5.69 (0.22)	5.63 (0.21)	0.20	0.82	0.964	0.996	0.907
Spatial span	4.40 (0.13)	4.33 (0.23)	4.93 (0.17)	3.67	*	0.086	0.059	0.988
Verbal fluency	26.85 (1.51)	29.25 (1.95)	40.15 (2.07)	15.35	***	***	***	0.757
Attentive matrices	35.96 (3.00)	44.55 (2.04)	46.79 (1.42)	6.69	**	**	0.869	*
TMT A	76.50 (13.86)	47.40 (8.44)	27.15 (2.61)	7.34	**	***	0.410	0.133
TMT B	141.43 (19.05)	102.44 (20.05)	73.40 (9.38)	5.29	**	**	0.376	0.319
TMT B-A	106.42 (14.84)	60.00 (12.15)	48.15 (7.43)	7.14	**	**	0.792	*
Stroop-reaction times	46.84 (7.15)	26.10 (5.06)	14.91 (1.62)	4.63	*	***	0.303	*
Stroop-errors	3.47 (1.54)	1.98 (0.79)	0.20 (0.18)	3.09	*	*	0.485	0.656
GDS	7.35 (1.25)	5.87 (0.81)	5.30 (0.94)	1.08	0.35	0.404	0.977	0.715

Results of the ANOVA model (F and *p*-values) and *post-hoc* comparisons with Sidak correction (*p*-values) between the groups are reported. Asterisks indicate significant differences ($^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$). MMSE, Mini-Mental State Examination; RCPM 47, Raven Progressive Colored Matrices; RAVLT, Rey Auditory Verbal Learning Task; ROCF, Rey-Osterrieth Complex Figure; TMT, Trail Making Test; GDS, Geriatric Depressive Scale.

algorithm implemented in Brain Vision Analyzer 2, applied over the whole continuous signal. Epochs were created starting from 200 ms before and lasting 800 ms after stimulus onset and baseline corrected from –200 ms to 0. All epochs were then visually inspected to discard those containing artifacts such as muscular activity, head movements or other sources of noise. The EEG was averaged separately for each target numerosity (from 1 to 6 targets) and target location (left or right hemifield). To obtain N2pc and CDA components, we computed the mean differential activity at posterior electrodes (PO7 and PO8, as in [17]) by subtracting the ipsilateral mean amplitude from the contralateral one with respect to the location of target presentation (left or right) and separately for each target numerosity. Contralateral and ipsilateral mean amplitude values were obtained collapsing the activity of the electrodes across target sites (PO7 was considered contralateral for right targets and ipsilateral for left ones; PO8 was considered contralateral for left targets and ipsilateral for right ones). The N2pc and CDA amplitude values at posterior electrodes (PO7 and PO8) were computed by extracting the mean amplitude values in the time window ranging from 250 ms to 350 ms (N2pc) and from 450 ms to 800 ms (CDA) post-stimulus onset for each participant and each target numerosity. The mean number of averaged trials for each numerosity was 60.01 in the sample of AD patients, 51.53 in MCI patients, and 68.46 in the group of healthy elderly.

Statistical analysis

Behavioral (mean error rate) and electrophysiological data (N2pc and CDA mean amplitude) were analyzed by a generalized linear mixed model (GLMM) with "numerosity" (number of targets presented from 1 to 6) as the within-subject factor and "group" (HC, AD, and MCI) as the between-subject factor. Since the behavioral data violated the assumption of a normal distribution of the dependent variable due to positive skewness, we performed GLMMs with a log-link function for the gamma distributed mean error rate variable. Without loss of generality, a constant equal to 0.1 was added to the error rate variable in order to address zero values. For electrophysiological data, which were normally distributed, we performed GLMMs for the Gaussian distributed N2pc and CDA mean amplitude variables with an identity link function. To investigate a possible difference in terms of age and education among the three groups of participants, we performed an ANOVA model for "age" and "education" variables. As the results showed that the three groups were

not matched on age ($F_{(2,53)} = 7.78$, $p \leq 0.001$) and education ($F_{(2,53)} = 4.06$, $p < 0.05$), we introduced these variables as fixed factors. Goodness of fit of models was evaluated through Akaike information criterion (AIC) and Bayesian information criterion (BIC). *Post hoc* comparisons were performed with Sidak correction for multiple comparisons. For both behavioral and electrophysiological data analysis, among the several GLMMs performed, the models that best (lowest AIC and BIC) fit the data were the ones with "numerosity" and "group" as the main effects and "numerosity x group" as the interaction effect (behavioral data: AIC = 784.27, BIC = 806.58; N2pc: AIC = 1164.31, BIC = 1186.61; CDA: AIC = 1031.31, BIC = 1053.62). Since none of the best-fit models included the variable "age" or "education", no effect of these variables on the results is supposed. In addition, receiver operating characteristics (ROC) curves were computed for the electrophysiological component/s which showed a significant effect of group in the overall analysis (i.e., the CDA, see below). ROC curves were calculated considering the CDA amplitude value elicited when three targets were presented, as this numerosity represented the mean breaking point between subitizing and counting in the three groups of participants. As a measure of diagnostic performance we provided the area under the curve index (AUC; $0.5 < AUC < 1$, with one indicating perfect accuracy). Moreover, sensitivity and specificity indexes were provided to support the reliability of the CDA as an electrophysiological biomarker for distinguishing between healthy and pathological aging.

RESULTS

Behavioral results

The results showed a significant main effect of numerosity ($F_{(5,318)} = 135.497$, $p < 0.001$) with an increase in error rates as a function of target numerosity. The group of AD patients performed worse than both the groups of MCI patients and HC, and MCI patients performed worse than the group of HC, as revealed by a significant main effect of group ($F_{(2,318)} = 56.649$, $p < 0.001$). Importantly, results showed a significant interaction between numerosity and group ($F_{(10,318)} = 1.978$, $p < 0.05$). *Post-hoc* comparisons revealed that AD patients performed worse than HC for all target numerosities (1–6) (all $p < 0.05$), while MCI patients exhibited a decreased performance in respect to HC mainly for larger numerosities (2-4-5-6) (all $p < 0.05$). Considering larger numerosities (4-5-6), *post-hoc* results revealed a significant difference also between AD and MCI patients with AD patients less accurate in the enumeration performance (all $p < 0.05$).

In order to investigate the subitizing span (i.e., how many targets are processed simultaneously) we computed the "efficiency value" for each participant, considering the first target numerosity at which accuracy fell below 90% [35] and then multiplying it by the value of total accuracy across all target numerosities (as in [31]). To compare the mean efficiency values across the groups, we performed an ANOVA model for Gaussian distributed values. "Age" and "education" covariate-adjusted results showed a significant effect of group ($F_{(2,56)} = 3.91$, $p < 0.05$), indicating lower capacity limits in pathological aging (AD: mean = 2.44, SEM = 0.27; MCI: mean = 3.09, SEM = 0.26) than in healthy aging (mean = 3.99, SEM = 0.25). However, from *post-hoc* comparisons with Sidak correction, only the difference between HC and AD emerged as statistically significant ($p < 0.05$). To investigate the relationship between the neuropsychological profile and the behavioral performance, we calculated both Pearson's (r) and Spearman's (rho) correlation coefficients for the efficiency value and scores at each neuropsychological test. The results showed a significant correlation of the efficiency value with MMSE (Fig. 1) and with various neuropsychological tests (Table 2). Albeit our aim was to provide a purely exploratory picture about the relation between each single neuropsychological test/cognitive domain and the behavioral performance—for which no multiple correction is generally required [36]—the robustness of the results was confirmed after Benjamini-Hochberg [37] multiple comparison correction.

Electrophysiological results

N2pc

Table 3 reports mean amplitude values of N2pc component as a function of target numerosities in the three groups of participants. Results showed a significant main effect of numerosity ($F_{(5,318)} = 3.78$, $p \leq 0.01$) with an increase in mean N2pc amplitude as a function of target numerosity. No significant effect was found for "group" ($F_{(2,318)} = 2.80$, $p = 0.06$) and neither for the interaction between "numerosity" and group" ($F_{(10,318)} = 0.34$, $p = 0.97$). To disentangle how N2pc amplitude was modulated

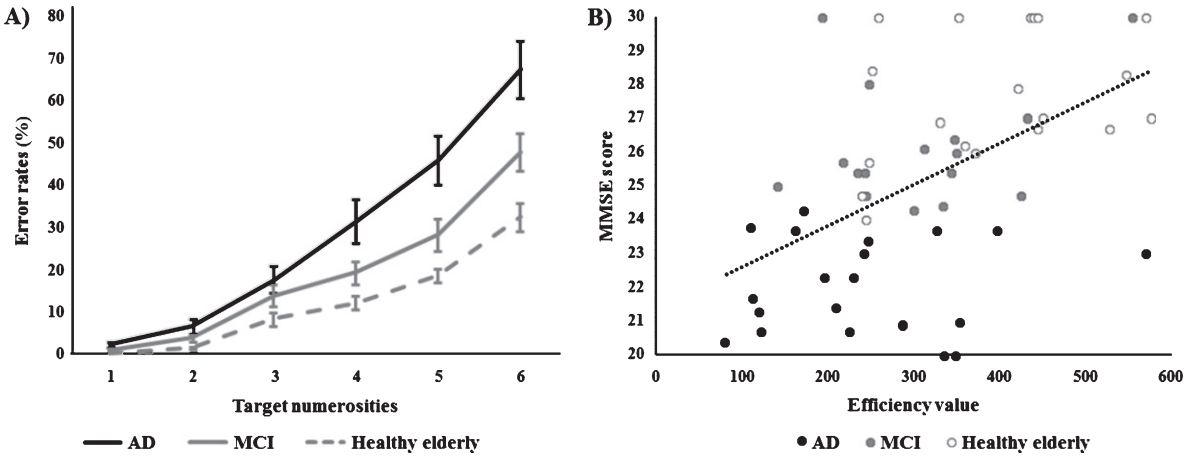

Fig. 1. Behavioral results of the multiple object processing task. A) Mean error rates as a function of target numerosities in the three groups of participants (AD, MCI, and HC). Error bars represent the standard error of the mean (±SEM). B) Scatter plot showing the significant correlation ($r = 0.52$, $p < 0.001$; rho = 0.55, $p < 0.001$) between the efficiency value and general cognitive abilities as assessed with the MMSE in the three groups of participants (AD, MCI, and HC).

Table 2
Pearson's (r) and Spearman (rho) correlation coefficients with associated *p*-values between the "efficiency value" and neuropsychological test scores (age- and education-adjusted) for AD patients, MCI and HC

	r	*p*	rho	*p*
MMSE	0.52	***	0.55	***
RCPM 47	0.48	***	0.46	***
RAVLT-immediate recall	0.43	***	0.43	***
RAVLT-delayed recall	0.48	***	0.48	***
Episodic memory	0.47	***	0.50	***
ROCF-copy	0.36	**	0.26	0.06
ROCF-recall	0.40	**	0.40	**
Digit Span	0.25	0.06	0.24	0.08
Spatial span	0.02	0.91	0.02	0.87
Verbal fluency	0.11	0.42	0.17	0.21
Attentive matrices	0.42	***	0.48	***
TMT A	–0.45	***	0.42	**
TMT B	–0.25	0.15	–0.25	0.14
TMT B-A	–0.25	0.10	–0.27	0.11
Stroop-reaction times	–0.54	***	–0.62	***
Stroop-errors	–0.46	***	–0.47	***
GDS	–0.21	0.12	–0.23	0.09

Asterisks indicate significant differences ($^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$).

by target numerosity, we computed the differences in amplitude between 3 versus 1 targets (small numerosities) and between 6 versus 4 targets (large numerosities) and compared them by a GLMM with "numerosity" (two levels: 3-1 and 6-4) as main effect. The results showed a significant effect of "numerosity" ($F_{(1,110)} = 7.62$, $p < 0.01$) with a higher amplitude difference ($p < 0.01$) for small numerosities (3-1: mean = –0.70, SEM = 0.14) as compared to large numerosities (6-4: mean = –0.09, SEM = 0.18). Figure 2 shows the grand-averaged N2pc component elicited by different target numerosity (from 1 to 6) in the three groups of participants.

CDA

Table 3 reports mean amplitude values of CDA component as a function of target numerosities in the three groups of participants. Results showed a significant main effect of "numerosity" ($F_{(5,318)} = 6.48$, $p < 0.001$) with an increase in mean CDA amplitude as a function of target numerosity. The significant main effect of "group" ($F_{(2,318)} = 9.57$, $p < 0.001$) revealed that AD patients (mean = –0.90, SEM = 0.10) showed an overall reduction of CDA amplitude as compared to MCI patients ($p < 0.001$; mean = –1.58, SEM = 0.12) and HC ($p = 0.06$; mean = –1.18, SEM = 0.10) whereas MCI patients showed an increased CDA amplitude compared to both AD patients ($p < 0.001$) and HC ($p < 0.05$). No significant effect was found for the interaction between "numerosity" and "group" ($F_{(10,318)} = 0.86$, $p = 0.57$). As for N2pc, we computed the differences in amplitude between 3 versus 1 (small numerosities) target and between 6 versus 4 targets (large numerosities) and compared them by a GLMM with "numerosity" (two levels: 3-1 and 6-4) as main effect. Results showed a significant effect of "numerosity" ($F_{(1,110)} = 6.25$, $p \leq 0.01$), with a higher amplitude difference ($p \leq 0.01$) for small numerosities (3-1: mean = –0.59, SEM = 0.16) as compared to large numerosities (6-4: mean = 0.01, SEM = 0.18). Figure 2 shows the grand-averaged CDA component

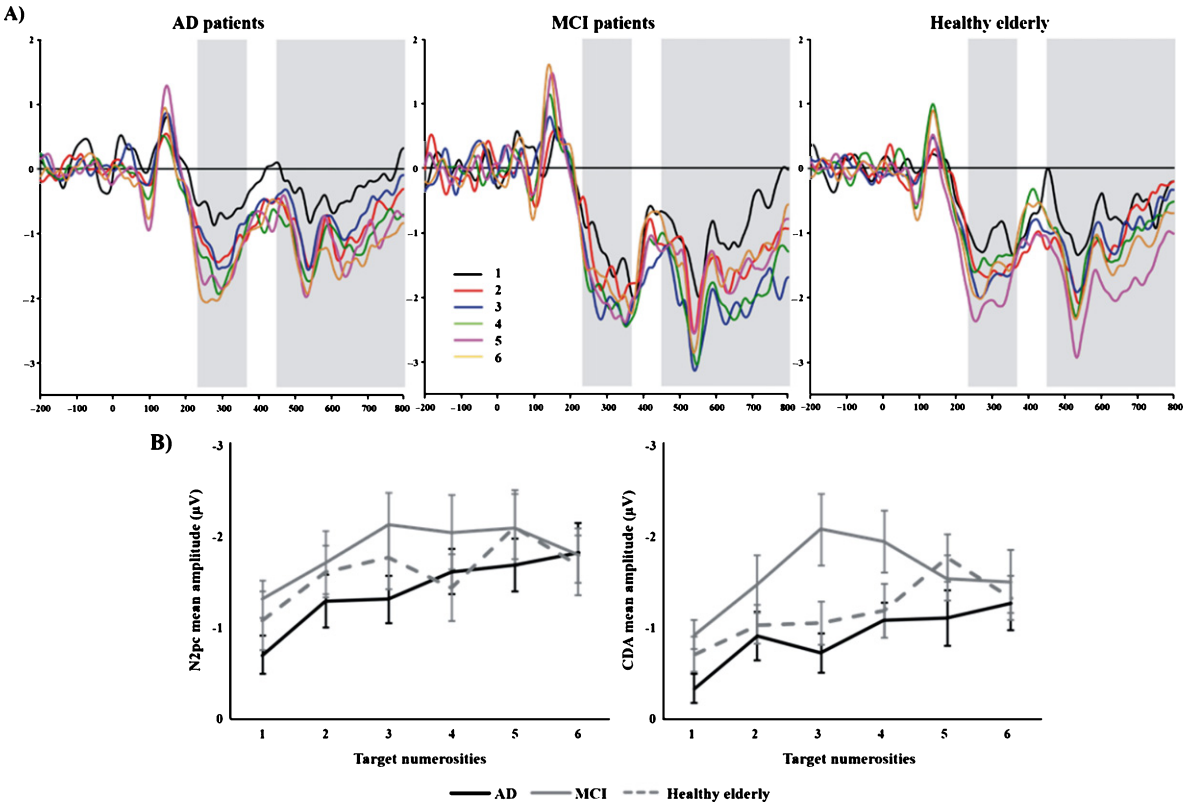

Fig. 2. Electrophysiological correlates of multiple object processing. A) Grand-averaged ERP waveforms for N2pc (250–350 ms) and CDA (450–800 ms) represented as a function of target numerosities in the three groups of participants (AD, MCI, and HC). For illustrative purposes only, data were filtered at 20 Hz. B) Mean amplitude values as a function of target numerosities in the three groups of participants (AD, MCI, and HC). Error bars represent the standard error of the mean (±SEM).

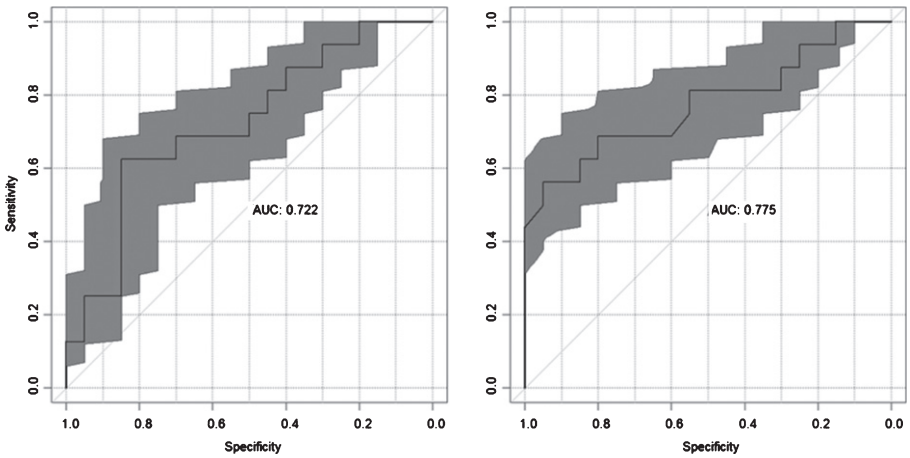

Fig. 3. Receiver operating characteristics curves (ROC) of CDA amplitude discriminating between HC and MCI patients (on the left; AUC = 0.72) and between MCI and AD patients (on the right; AUC = 0.78). The grey shadows around the ROC curve represent the confidence intervals.

elicited by different target numerosities (from 1 to 6) in the three groups of participants.

ROC curve analysis (see Fig. 3) revealed moderate predictive performance of CDA amplitude in discriminating between HC and MCI patients (AUC = 0.72). Considering a CDA amplitude value of –1.66 μV as cut-off score, specificity and sensitivity indexes were 0.85 and 0.63, respectively. In discriminating between MCI and AD patients, CDA showed good predictive performance (AUC = 0.78), with specificity and sensitivity indexes of 0.80 and 0.69, respectively, when considering a cut-off score of –1.46 μV.

DISCUSSION

The results of the present study shed new light on the behavioral and electrophysiological mechanisms responsible for the MOP impairment during enumeration in AD and, for the first time, revealed how this process is affected in the prodromal stage of the disease, namely MCI.

Considering the behavioral performance, AD patients showed an overall decline in enumeration abilities, with an increase in error rates that encompasses all target numerosities and a reduction of the subitizing span. These results are in line with previous studies, which revealed a decline of the subitizing span that increases with disease progression [27–29]. Previous studies reported that AD patients were overall slower in the enumeration performance, but not less accurate [28, 29]. In contrast, the present results pointed out a decrease in accuracy that overall encompasses all target numerosity. This discrepant finding is likely due to methodological differences between the studies. Stimulus duration determines whether target numerosity mainly affects accuracy (brief duration, e.g., [32]) or response times (unlimited duration, e.g., [38]). In line with this observation, in the present study the duration of the stimulus array was brief (i.e., 400 ms), whereas in previous studies it was presented until the patient's response. Whereas in AD both subitizing and counting were affected by the disease, in the prodromal stage only counting was found to decline, with preserved accuracy for small numerosities within the subitizing range. Evidence of impaired numerical abilities in prodromal patients has already been highlighted in studies [39] adopting a paper-and-pencil subtest of the Numerical Activities of Daily Living battery [40] which consisted in comparing the number of stimuli in two displays presented simultaneously. The fact that MCI patients showed a relatively preserved performance for small target quantities is in line with previous studies reporting that AD impairs enumeration abilities in the subitizing range only in a later stage of the disease [28, 29]. Considering the variability in the diagnostic criteria, it is reasonable that the disease severity of some MCI patients may overlap with that of the patients considered as mild AD patients in the study by Maylor and colleagues [29]. The significant correlation between the subitizing span and MMSE further substantiate previous results on the relationship between the ability to process multiple objects and the severity of the disease [27, 28]. Enumeration performance also correlated with various neuropsychological tests mainly in the domain of attention (attentive matrices, TMT A, and Stroop test) and memory (verbal memory: RAVLT immediate and delayed, episodic memory; visuo-spatial memory: ROCF recall), supporting the view that the ability to process multiple objects simultaneously is linked to a variety of cognitive functions mainly in the visuo-spatial domain. Therefore, this finding suggests that the decline in subitizing span observed in AD pathology is expressed as a continuum, such that the ability to simultaneously process multiple objects decreases as a function of cognitive impairment, with more impaired patients having the most reduced subitizing span.

The EEG results allowed us to delineate the neural dynamics associated with the behavioral impairment and the specific involvement of attention and VSTM mechanisms in this decline. The early individuation component (N2pc) was preserved both in AD and in MCI patients, whereas the later component linked to visual VSTM abilities (CDA) showed a distinctive

Table 3
Mean amplitude values (±SEM) of N2pc and CDA components as a function of target numerosities in the three groups of participants (AD, MCI, and HC)

	AD	MCI	HC
N2pc			
1	–0.70 (0.25)	–1.32 (0.28)	–1.07 (0.25)
2	–1.29 (0.29)	–1.71 (0.33)	–1.61 (0.29)
3	–1.31 (0.31)	–2.13 (0.35)	–1.77 (0.31)
4	–1.61 (0.33)	–2.05 (0.37)	–1.44 (0.33)
5	–1.68 (0.34)	–2.10 (0.38)	–2.11 (0.34)
6	–1.82 (0.32)	–1.79 (0.35)	–1.68 (0.32)
CDA			
1	–0.32 (0.17)	–0.92 (0.19)	–0.86 (0.17)
2	–0.91 (0.25)	–1.47 (0.28)	–1.45 (0.25)
3	–0.72 (0.27)	–2.08 (0.30)	–1.05 (0.27)
4	–1.07 (0.27)	–1.94 (0.30)	–1.19 (0.27)
5	–1.10 (0.27)	–1.54 (0.30)	–1.77 (0.27)
6	–1.27 (0.28)	–1.50 (0.32)	–1.32 (0.28)

altered pattern in the groups of patients as compared to HC.

To our knowledge, this is the first study investigating the N2pc component in a sample of AD patients. The results showed that its amplitude was equally modulated by target numerosity in all three groups, suggesting that the attentional selection/individuation mechanisms reflected by N2pc are not affected by pathological aging (neither in MCI nor in mild AD). Thus, the current results suggest that N2pc cannot be considered a good candidate for a diagnostic marker to distinguish between healthy and pathological aging. However, the patients enrolled in the present study were in the early (mild AD) and in the prodromal phase (MCI) of the disease; therefore, a decline in the N2pc component in the later stages of the disease (i.e., moderate AD) cannot be ruled out. Contrary to the present finding, Cespon and colleagues [41, 42] reported a reduction of N2pc amplitude in multi-domain amnestic MCI patients as compared to healthy elderly, suggesting the utility of N2pc to early identify a specific MCI subtype. The discrepant finding may be due to the experimental differences between the studies. In fact, the study by Cespon used a Simon task, which involves different visuospatial processing and related neural dynamics as compared to our enumeration paradigm. Additionally, healthy elderly may perform better than MCI a Simon task in which only one element at a time needs to be processed. In our task, instead, most of the targets are multiple objects, possibly leading to an impaired performance even of healthy participants and thus attenuating the differences with MCI. Results on CDA component showed an overall alteration of CDA amplitude in pathological aging, with a specific pattern that differed according to the stage of the disease. The reliability of CDA as an index to distinguish between healthy and pathological aging was confirmed by the ROC curve analysis. This analysis showed a good ability in discriminating the different stages along the continuum of disease severity, and in distinguishing healthy elderly from patients in the prodromal phase.

The results on AD patients indicated a global reduction of CDA amplitude, which correlated with an overall decline in multiple object processing for both small (subitizing) and large (counting) target numerosities. Conversely, MCI patients showed an increased CDA amplitude concomitantly with a relatively preserved behavioral performance. These CDA alterations are ascribable to the well-known impairment of short-term memory abilities in AD [43] and even in its prodromal stage [44–46]. In addition, the relevance of the VSTM component in the present study may have been strengthened by the use of time-limited display presentations. As mentioned above, the fact that visual stimuli were briefly presented for 400 ms did not allow participants to count the targets while they were still visible, and may have forced them to rely on the memory trace of the stimulus, thus requiring more working memory resources to elaborate that trace. We speculate that in a demanding context where targets are presented among distractors, the VSTM resources in AD patients are not sufficient to maintain the memory trace of the items previously individuated, even when only few elements are presented. Consistently, the pathophysiology underlying AD induced a reduction of CDA amplitude during the execution of the enumeration task.

The amplitude increase of CDA observed in MCI patients may be interpreted as a compensatory mechanism. This mechanism may allow MCI patients to partially overcome the cognitive decline by overactivating the neural areas involved in the active maintenance of the relevant elements during the enumeration task. Indeed, the overactivation was associated with a good level of performance in the condition with small target numerosities (subitizing range). In contrast, there was a breakdown in the performance for larger target quantities (counting range), possibly due to the limited available neural resources to support the execution of the enumeration task in more demanding conditions. However, a potential limit of this compensatory interpretation is represented by the lack of significant correlation between the behavioral performance and CDA amplitude. Future studies with higher power might overcome this shortcoming.

These results may be interpreted in the framework of the "Compensation-related utilization of neural circuits" (CRUNCH; [47]) in the aging brain. The CRUNCH model postulates that, in conditions of low cognitive load, elderly recruit more neural resources than younger adults when their performance is equivalent. For more difficult contexts (high cognitive load) the compensatory mechanisms vanish, leading to insufficient recruitment of neural resources and to a decline in performance. The aging brain can recruit additional neural resources to uphold some cognitive functions, but this compensatory mechanism will be no longer effective when the limit of the (overall reduced) available resources is reached [47].

Although CRUNCH and other compensation models have compared healthy elderly with young adults, several studies suggest that the mechanisms of compensation derived from healthy aging can be applied to pathological aging too (for a review, see [48]).

The peculiar pattern of CDA alteration found in the present study resonates with the results of previous neuroimaging studies on AD and MCI. For example, investigating how memory networks are affected by AD pathophysiology (for a review see [49]), Celone and colleagues [50] hypothesized a nonlinear pattern of activation across the stages of AD continuum, ranging from hyperactivation in the earlier prodromal stages of MCI to hypoactivation as the disease severity advanced to mild AD. Accordingly, fMRI data comparing hippocampal activation across the continuum of healthy aging, MCI and mild AD, revealed that less impaired MCI patients had greater hippocampal activation as compared to healthy elderly, whereas more impaired MCI patients showed a decrease in the activation, similar to the one emerged in AD patients [50].

In the context of EEG studies, parieto-occipital compensatory recruitment in MCI patients has been reported as an abnormal enhancement of P450 amplitude over posterior regions during a memory task, indicating that MCI patients need to recruit additional resources in order to carry out the task [51]. Crucially, this compensatory mechanism has been observed only in the prodromal phase of AD but not in the later stages when the diagnosis of AD had taken hold [51]. In line with this result, in the present study we found that the supposed compensatory mechanism exhibited as a more pronounced CDA component in MCI patients was no longer present in AD patients, who conversely showed a suppression of the aforementioned component. Thus, the severe neurodegeneration of the dementia phase may prevent the recruitment of additional neural resources, and may not allow compensatory mechanisms to take place as the pathological burden becomes more severe [51, 52]. These conclusions are consistent with the findings of functional activation studies reviewed by Prvulovic and colleagues [53], who demonstrated that the progression of neural degeneration can lead to phenomena of either hyper-activation (usually associated with mildly impaired performance, as in the case of MCI patients in our study), or hypo-activation, which is linked to a greater impairment in the performance (as showed here for AD patients).

All this evidence supports the idea that CDA may be a useful neural signature not only to distinguish between healthy and pathological aging, but also to characterize the different stages along the AD continuum. This finding is substantiated by a previous EEG study on MOP in elderly considered at-risk to develop MCI, who showed changes in the CDA component with respect to healthy controls, suggesting that this neural signature may be particularly sensitive also to the preclinical stage of disease progression [54].

Conclusions

The present results suggested that MOP could be a promising paradigm to identify both behavioral and neural markers for a distinction between the different stages along the AD continuum, starting from the prodromal MCI phase. Regarding the behavioral results, the impairment in MOP ranges from an initial decline visible only for more demanding target numerosities (counting range) in the prodromal phase of the disease (MCI), to a decline that encompasses both counting and subitizing (small target quantities) processes in the earlier stage of the disease (mild AD). The neural dynamics underlying these deficits are associated to changes in VSTM mechanisms, as indexed by the alteration of CDA activation both in mild AD and in MCI patients. This alteration follows a non-linear pathway along the continuum of the disease: whereas in the prodromal phase there is a more pronounced CDA (presumably indexing a compensatory mechanism), in the mild stage of AD there is a reduction of its amplitude. Together, these results disclosed that neural signature of VSTM, namely CDA, may represent a valid index to distinguish between healthy and pathological aging. While the approach used and the results in this study are not sufficient to establish CDA as a reliable biomarker of AD pathology, they lay the foundation for further research in this direction.

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REFERENCES

[1] Ferri CP, Prince MP, Brayne C, Brodaty H, Fratiglioni L, Ganguli M, Hall K, Hasegawa K, Hendrie H, Huang Y, Jorm A, Mathers C, Menezes PR, Rimmer E, Scazufca M (2005) Global prevalence of dementia: A Delphi consensus study. *Lancet* **366**, 2112-2117.

[2] Hardy J, Selkoe DJ (2002) The amyloid hypothesis of Alzheimer's disease: Progress and problems on the road to therapeutics. *Science* **297**, 353-356.

[3] Jack CRJ, Knopman DS, Jagust WJ, Shaw LM, Aisen PS, Weiner W, Petersen RC, Trojanowski JQ (2010) Hypothetical model of dynamic biomarkers of the Alzheimer's pathological cascade. *Lancet Neurol* **9**, 1-20.

[4] Dubois B, Feldman HH, Jacova C, Cummings JL, DeKosky ST, Barberger-Gateau P, Delacourte A, Frisoni G, Fox NC, Galasko D, Gauthier S, Hampel H, Jicha GA, Meguro K, O'Brien J, Pasquier F, Robert P, Rossor M, Salloway S, Sarazin M, de Souza LC, Stern Y, Visser PJ, Scheltens P (2010) Revising the definition of Alzheimer's disease: A new lexicon. *Lancet Neurol* **9**, 1118-1127.

[5] Petersen R, Smith G, Waring S, Ivnik R, Tangalos E, Kokmen E (1999) Mild cognitive impairment: Clinical characterization and outcome. *Arch Neurol* **56**, 303-308.

[6] Petersen RC (2004) Mild cognitive impairment as a clinical entity and treatment target. *Arch Neurol* **62**, 1160-1163; discussion 1167.

[7] Chapman RM, Mccrary JW, Gardner MN, Sandoval TC, Guillily MD, Reilly LA, Degrush E (2011) Brain ERP components predict which individuals progress to Alzheimer's disease and which do not. *Neurobiol Aging* **32**, 1742-1755.

[8] Chapman RM, Nowlis GH, McCrary JW, Chapman JA, Sandoval TC, Guillily MD, Gardner MN, Reilly LA (2007) Brain event-related potentials: Diagnosing early-stage Alzheimer's disease. *Neurobiol Aging* **28**, 194-201.

[9] Mazza V, Brignani D (2016) Electrophysiological advances on multiple object processing in aging. *Front Aging Neurosci* **8**, 46.

[10] Ansari D, Lyons IM, van Eimeren L, Xu F (2007) Linking visual attention and number processing in the brain: The role of the temporo-parietal junction in small and large symbolic and nonsymbolic number comparison. *J Cogn Neurosci* **19**, 1845-1853.

[11] Vetter P, Butterworth B, Bahrami B (2011) A candidate for the attentional bottleneck: Set-size specific modulation of the right TPJ during attentive enumeration. *J Cogn Neurosci* **23**, 728-736.

[12] Vuokko E, Niemivirta M, Helenius P (2013) Cortical activation patterns during subitizing and counting. *Brain Res* **1497**, 40-52.

[13] Finke K, Myers N, Bublak P, Sorg C (2013) A biased competition account of attention and memory in Alzheimer's disease. *Philos Trans R Soc Lond B Biol Sci* **368**, 20130062.

[14] Jagust W, Reed B, Mungas D, Ellis W, DeCarli C (2007) What does fluorodeoxyglucose PET imaging add to a clinical diagnosis of dementia? *Neurology* **69**, 871-877.

[15] Kaufman E, Lord M, Reese T, Volkmann J (1949) The discrimination of visual number. *Am J Psychol* **62**, 498-525.

[16] Mazza V, Caramazza A (2015) Multiple object individuation and subitizing in enumeration: A view from electrophysiology. *Front Hum Neurosci* **9**, 1-7.

[17] Pagano S, Fait E, Monti A, Brignani D, Mazza V (2015) Electrophysiological correlates of subitizing in healthy aging. *PLoS One* **10**, e0131063.

[18] Eimer M (1996) The N2pc component as an indicator of attentional selectivity. *Electroencephalogr Clin Neurophysiol* **99**, 225-234.

[19] Luck SJ, Hillyard SA (1994) Electrophysiological correlates of feature analysis during visual search. *Psychophysiology* **31**, 291-308.

[20] Vogel EK, Machizawa MG (2004) Neural activity predicts individual differences in visual working memory capacity. *Nature* **428**, 748-751.

[21] Berggren N, Eimer M (2016) Does contralateral delay activity reflect working memory storage or the current focus of spatial attention within visual working memory? *J Cogn Neurosci* **28**, 2003-2020.

[22] Mazza V, Caramazza A (2012) Perceptual grouping and visual enumeration. *PLoS One* **7**, 1-7.

[23] Peterson DJ, Gözenman F, Arciniega H, Berryhill ME (2015) Contralateral delay activity tracks the influence of Gestalt grouping principles on active visual working memory representations. *Atten Percept Psychophys* **77**, 2270-2283.

[24] Mazza V, Turatto M, Umiltá C, Eimer M (2007) Attentional selection and identification of visual objects are reflected by distinct electrophysiological responses. *Exp Brain Res* **181**, 531-536.

[25] Mazza V, Caramazza A (2011) Temporal brain dynamics of multiple object processing: The flexibility of individuation. *PLoS One* **6**, e17453.

[26] Ester EF, Drew T, Klee D, Vogel EK, Awh E (2012) Neural measures reveal a fixed item limit in subitizing. *J Neurosci* **32**, 7169-7177.

[27] Nebes RD, Brady CB, Reynolds CF 3rd (1992) Cognitive slowing in Alzheimer's disease and geriatric depression. *J Gerontol* **47**, 331-336.

[28] Maylor EA, Watson DG, Muller Z (2005) Effects of Alzheimer's disease on visual enumeration. *J Gerontol B Psychol Sci Soc Sci* **60**, 129-135.

[29] Maylor EA, Sheehan B, Watson DG, Henderson EL (2008) Enumeration in Alzheimer's disease and other late life psychiatric syndromes. *Neuropsychologia* **46**, 2696-2708.

[30] McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM (1984) Clinical diagnosis of Alzheimer's disease: Report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. *Neurology* **34**, 939-944.

[31] Pagano S, Mazza V (2012) Individuation of multiple targets during visual enumeration: New insights from electrophysiology. *Neuropsychologia* **50**, 754-761.

[32] Pagano S, Lombardi L, Mazza V (2014) Brain dynamics of attention and working memory engagement in subitizing. *Brain Res* **1543**, 244-252.

[33] Crawford TJ, Higham S, Mayes J, Dale M, Shaunak S, Lekwuwa G (2013) The role of working memory and attentional disengagement on inhibitory control: Effects of aging and Alzheimer's disease. *Age (Omaha)* **35**, 1637-1650.

[34] Danckert J, Maruff P, Crowe S, Currie J (1998) Inhibitory processes in covert orienting in patients with Alzheimer's disease. *Neuropsychology* **12**, 225-241.

[35] Franconeri SL, Alvarez GA, Enns JT (2007) How many locations can be selected at once? *J Exp Psychol Hum Percept Perform* **33**, 1003-1012.

[36] Bender R, Lange S (2001) Adjusting for multiple testing–when and how? *J Clin Epidemiol* **54**, 343-349.

[37] Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: A practical and powerful approach to multiple testing. *J R Stat Soc B* **57**, 289-300.

[38] Trick LM, Pylyshyn ZW (1993) What enumeration studies can show us about spatial attention: Evidence for limited capacity preattentive processing. *J Exp Psychol Hum Percept Perform* **19**, 331-351.

[39] Benavides-Varela S, Burgio F, Meneghello F, De Marco M, Arcara G, Rigon J, Pilosio C, Butterworth B, Venneri A, Semenza C (2015) Anatomical substrates and neurocognitive predictors of daily numerical abilities in mild cognitive impairment. *Cortex* **71**, 58-67.

[40] Semenza C, Meneghello F, Arcara G, Burgio F, Gnoato F, Facchini S, Benavides-Varela S, Clementi M, Butterworth B (2014) A new clinical tool for assessing numerical abilities in neurological diseases: Numerical activities of daily living. *Front Aging Neurosci* **6**, 112.

[41] Cespòn J, Galdo-Alvarez S, Diaz F (2013) Electrophysiological correlates of amnestic mild cognitive impairment in a simon task. *PLoS One* **8**, e81506.

[42] Cespon J, Galdo-Alvarez S, Pereiro AX, Diaz F (2015) Differences between mild cognitive impairment subtypes as indicated by event-related potential correlates of cognitive and motor processes in a Simon task. *J Alzheimers Dis* **42**, 631-647.

[43] Baddeley AD, Bressi S, Della Sala S, Logie R, Spinnler H (1991) The decline of working memory in Alzheimer's disease. *Brain* **114**, 2521-2542.

[44] Brandt J, Aretouli E, Neijstrom E, Samek J, Manning K, Albert MS, Bandeen-Roche K (2009) Selectivity of executive function deficits in mild cognitive impairment. *Neuropsychology* **23**, 607-618.

[45] Saunders NLJ, Summers MJ (2011) Longitudinal deficits to attention, executive, and working memory in subtypes of mild cognitive impairment. *Neuropsychology* **25**, 237-248.

[46] Saunders NLJ, Summers MJ (2010) Attention and working memory deficits in mild cognitive impairment. *J Clin Exp Neuropsychol* **32**, 350-357.

[47] Reuter-Lorenz PA, Cappell KA (2008) Neurocognitive aging and compensation hypothesis. *Curr Dir Psychol Sci* **17**, 177-182.

[48] Scheller E, Minkova L, Leitner M, Kloppel S (2014) Attempted and successful compensation in preclinical and early manifest neurodegeneration – a review of task fMRI studies. *Front Psychiatry* **5**, 1-16.

[49] Chhatwal J, Sperling R (2013) Functional MRI of mnemonic networks across the spectrum of normal aging, mild cognitive impairment and Alzheimer's disease. *J Alzheimers Dis* **31**, S155-S167.

[50] Celone KA, Calhoun VD, Dickerson BC, Atri A, Chua EF, Miller SL, DePeau K, Rentz DM, Selkoe DJ, Blacker D, Albert MS, Sperling RA (2006) Alterations in memory networks in mild cognitive impairment and Alzheimer's disease: An independent component analysis. *J Neurosci* **26**, 10222-10231.

[51] Beuzeron-Mangina H, Mangina CA (2009) Excessive compensatory recruitment as a compulsory neurophysiological mechanism in very early Alzheimer's disease as compared to mild vascular dementia and to age-matched normal controls. *Int J Psychophysiol* **73**, 164-169.

[52] Buckner RL (2004) Memory and executive function in aging and AD: Multiple factors that cause decline and reserve factors that compensate. *Neuron* **44**, 195-208.

[53] Prvulovic D, Van De Ven V, Sack AT, Maurer K, Linden DEJ (2005) Functional activation imaging in aging and dementia. *Psychiatry Res* **140**, 97-113.

[54] Newsome RN, Pun C, Smith VM, Ferber S, Barense MD (2013) Neural correlates of cognitive decline in older adults at-risk for developing MCI: Evidence from the CDA and P300. *Cogn Neurosci* **4**, 152-162.