Using visual evoked potentials for the early detection of amnestic mild cognitive impairment: a pilot investigation

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Objective: Amnesic mild cognitive impairment (MCIa) is often characterized as an early stage of Alzheimer’s dementia (AD). The latency of the P2, an electroencephalographic component of the flash visual evoked potential (FVEP), is significantly longer in those with AD or MCIa when compared with controls. The present investigation examined the diagnostic accuracy of several FVEP-P2 procedures in distinguishing people with MCIa and controls.

Methods: The latency of the FVEP-P2 was measured in participants exposed to a single flash condition and five double flash conditions. The double flash conditions had different inter-stimulus intervals between the pair of strobe flashes.

Results: Significant group differences were observed in the single flash and two of the double flash conditions. One of the double flash conditions (100 ms) displayed a higher predictive accuracy than the single flash condition, suggesting that this novel procedure may have more diagnostic potential. Participants with MCIa displayed similar P2 latencies across conditions, while controls exhibited a consistent pattern of P2 latency differences. These differences demonstrate that the double stimulation procedure resulted in a measurable refractory effect for controls but not for those with MCIa.

Conclusions: The pattern of P2 group differences suggests that those with MCIa have compromised cholinergic functioning that results in impaired visual processing. Results from the present investigation lend support to the theory that holds MCIa as an intermediate stage between normal healthy aging and the neuropathology present in AD. Measuring the FVEP-P2 during several double stimulation conditions could provide diagnostically useful information about the health of the cholinergic system.

Key words: mild cognitive impairment; MCI; AD; FVEP; P2; flash visual evoked potential; Alzheimer; acetylcholine

Introduction

Mild cognitive impairment (MCI) has gained wide acceptance as a term referring to various forms of cognitive decline that are not attributable to normal, healthy aging (Petersen, 2004). The amnestic subtype of MCI (MCIa), which is characterized by a deficit in episodic memory but the preservation of general cognitive functioning and the absence of dementia (Petersen, 2004; Levey et al., 2006), may be a transitional period between normal aging and more serious pathological conditions, such as Alzheimer’s dementia (AD; Grundman, et al., 2004; Levey, et al., 2006). Conversion from MCIa into AD has been demonstrated to occur for about 10–15% per year for patients diagnosed with MCIa as compared with 1–2% per year for healthy, age-matched controls (Gauthier, et al., 2006).
Based on the high rates of conversion for MCIa to AD, researchers have studied MCIa with the hope that an early diagnosis of AD would lead to an increase in potential for treatment options that may delay or even prevent further cognitive decline (Bischkopf et al., 2002). While some progress has been made in developing methods of early detection, very few have proven to be diagnostically useful (Grundman, et al., 2004; Petersen, 2004; Levey, et al., 2006).

One of the earliest neurophysiological changes seen in AD involves a reduction of cholinergic functioning (Moore, 1997). Acetylcholine is a neurotransmitter that plays an important role in cognitive functioning, including memory and some types of sensory processing (Bajalan et al., 1986). It has been hypothesized that the cognitive deficits associated with MCIa and AD are a direct result of the impaired functioning of the brain’s cholinergic systems (Bajalan et al., 1986; Reeves, et al., 1999; Gron, et al., 2006; Herholz et al., 2008). Therefore, reliable and valid measures of early visual processing could, in theory, be used to assess the integrity of an individual’s cholinergic system and serve as a clinical diagnostic tool when diagnosing MCIa and AD (Givre et al., 1994; Mielke et al., 1995).

Since the early 1990s, research has focused on developing a noninvasive method of assessing cholinergic functioning that could be used to improve the diagnostic accuracy of the more commonly used neuropsychological test battery (Moore, 1997; Babiloni et al., 2004; Moretti et al., 2004; Babiloni et al., 2009). A detachment of this research has focused on the early visual processing deficits seen in AD and found robust statistically significant group differences in evoked potential latencies to simple flash stimuli, but not to pattern reversal stimuli. This pattern of results suggests that the luminance channel of the human visual system is heavily innervated by cholinergic neurons that may be adversely impacted early in the AD disease process.

By measuring the performance of the luminance channel, it may be possible to reliably gauge the health of the brain’s cholinergic system. One of the best methods for accomplishing this is with the P2 component of the flash visual evoked potential (FVEP-P2; Coburn, et al., 2005). The P2 is the second positive wave of the visual evoked potential (VEP) detectable with electroencephalography (EEG) and is a prominent feature of early cortical processing. It has been demonstrated repeatedly that people with MCIa and AD exhibit a selective delay in the FVEP-P2 latency while showing no such differences in response to other types of visual stimuli (Swanwick, et al., 1996; Moore, 1997; Coburn, et al. 2003). Despite this consistent pattern of group differences, individual variability contributes to overlapping FVEP-P2 latency distributions between normal, MCIa, and AD groups that reduces the sensitivity and specificity of the FVEP-P2 when used as a clinical diagnostic tool. Consequently, a method of further separating the distributions of FVEP-P2 latencies associated with these groups must be developed in order to increase the sensitivity and specificity of the FVEP-P2 if it is to be used clinically.

One possible modification to the FVEP procedure might be the use of a double flash stimulation, which has been used previously to study the refractory period of the human visual system (Musselwhite & Jeffreys, 1983; Skrandies & Raile, 1989). VEP responses to single and double stimulations have been compared as a way to measure how quickly the visual system could recover from a single flash stimulus. Presenting successive flash stimuli, while varying the inter-stimulus interval (ISI) of each stimulus pair, resulted in little or no response to the second flash stimulus in conditions with an ISI of 50 milliseconds (ms) or less (Skrandies & Raile, 1989). However, a small response was observed in conditions with an ISI longer than the refractory threshold of 50 ms, and it became more robust as the ISI increased in length.

The double flash procedure has yet to be studied in patients with either MCIa or AD. Given the early loss of cholinergic neurons in patients with MCIa and AD, the double stimulation technique may prove useful in further separating normal healthy controls and patients with either MCIa or AD. For example, one might expect the first flash to tax an already compromised cholinergic visual system, leading to an abnormally delayed response to the second flash stimulus.

The purpose of the current study was to evaluate whether a novel double stimulation procedure could be used to distinguish people with MCIa from a group of healthy controls. It was predicted that the P2 latencies of MCIa patients would be significantly longer, on average, than the FVEP-P2 latencies of healthy controls, especially for ISIs approaching the refractory period. Finally, it was predicted that a longer refractory effect would be observed for individuals with MCIa than controls.

**Methods**

**Participants**

Participants were recruited from a neuropsychology clinic and a mid-sized, southeastern university. Final analyses were conducted on a clinical group of five (mean age, 78.00 years, $SD = 10.30$) and a control group of eight (mean age, 65.57 years, $SD = 7.91$).
Ten participants from the neuropsychology clinic and three participants from the university were recruited because of self-reported memory problems and received the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS). Five of the 13 referrals were not found to have MCIa. The MCIa clinical guidelines set up by Albert et al. (2011) were used to determine who would qualify as a patient with MCIa. Participants who scored more than 1.5 standard deviations below the mean on either immediate or delayed memory, while scoring within normal ranges on all other subscales, including visuospatial, language, and attention, qualified as MCIa (Table 1). Those who were seen for memory problems, but scored within normal limits on all other cognitive domains, were used as healthy controls. Each participant’s medical history was examined to ensure that participants were free of significant neurological or psychiatric histories and other health problems that were likely to affect brain activity or place them at risk for seizures. Patients and controls were also screened for medications that have significant effects on the cholinergic system, such as acetylcholinesterase inhibitors. Each person was paid between 20 and 50 dollars for their participation, depending on the time spent driving to the clinic or laboratory.

Materials

The RBANS is composed of five subscales designed to assess the cognitive functioning of patients within four cognitive domains: attention, visuospatial, language, and memory (immediate and delayed). For standardization purposes, subtest scores are scaled differently depending on the participants’ age. Each subtest score is age-corrected to be standardized with a mean of 100 and a standard deviation of 15.

Evidence for the test–retest reliability of the RBANS comes from Wilks et al. (2002), who found a mean intraclass correlation coefficient of 0.77 for the RBANS total score, indicating adequate test–retest reliability. Further evidence for the validity of the RBANS comes from a study conducted by Randolph, et al. (1998), who demonstrated the clinical utility of the RBANS in identifying both normal and impaired cognitive functioning in older adults. Duff et al. (2008) reproduced the aforementioned results, demonstrating the high diagnostic accuracy of both the immediate (98%) and delayed (96%) memory components of the RBANS in identifying those with AD.

Apparatus

The EEG signals were amplified by four Biopac (Biopac Systems Inc., Holliston, MA, USA) ERS100C EEG amplifiers (60 Hz upper limit band-pass filter) and recorded by the Biopac MP150 Data Acquisition System (Biopac Systems Inc., Holliston, MA, USA). Three shielded electrodes were used for the active sites, referenced to two unshielded clip electrodes placed on the left and right earlobes. Eye-movement artifact was measured using two shielded electrodes that were placed above and below the right eye. The Biopac Acqknowledge (Biopac Systems Inc., Holliston, MA, USA) software package was used to record and average each EEG signal and also to control the flash stimulation of the Grass model PS 33 Plus photostimulator. Data were analyzed statistically with the SPSS 17.0 (SPSS Inc., Chicago, IL, USA) software package.

Procedure

Participants recruited from the neuropsychology clinic were administered the RBANS and the novel EEG procedure at the clinic. Participants recruited from the university were administered the RBANS and the novel EEG procedure in the Cognitive Neuroscience Laboratory at the University of West Florida. The same procedures and apparatus were used at both

Table 1  Demographic information for MCIa patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 8; 4 females)</th>
<th>MCIa (n = 5; 4 females)</th>
<th>F</th>
<th>p</th>
<th>Partial η²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>M 65.57, SD 7.91</td>
<td>M 78.00, SD 10.30</td>
<td>5.63</td>
<td>0.039</td>
<td>0.36</td>
</tr>
<tr>
<td>RBANS</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Immediate memory</td>
<td>M 98.50, SD 14.49</td>
<td>M 70.20, SD 15.26</td>
<td>11.29</td>
<td>0.006</td>
<td>0.51</td>
</tr>
<tr>
<td>Delayed memory</td>
<td>M 100.50, SD 10.57</td>
<td>M 59.80, SD 9.01</td>
<td>50.47</td>
<td>&lt;0.001</td>
<td>0.82</td>
</tr>
<tr>
<td>Visuospatial</td>
<td>M 103.88, SD 10.87</td>
<td>M 92.80, SD 5.26</td>
<td>4.43</td>
<td>0.059</td>
<td>0.29</td>
</tr>
<tr>
<td>Language</td>
<td>M 99.13, SD 10.12</td>
<td>M 100.00, SD 16.43</td>
<td>0.01</td>
<td>0.907</td>
<td>0.00</td>
</tr>
<tr>
<td>Attention</td>
<td>M 100.38, SD 8.90</td>
<td>M 96.60, SD 11.89</td>
<td>0.43</td>
<td>0.525</td>
<td>0.04</td>
</tr>
</tbody>
</table>

MCIa, amnesic mild cognitive impairment; RBANS, Repeatable Battery for the Assessment of Neuropsychological Status.
research locations. After completing the informed consent form, participants were seated in a comfortable chair while EEG electrodes were placed at the target sites on the scalp. All electrodes were placed according to a modified 10–20 international system of electrode placement (Coburn et al., 2005). Electrodes were placed at recording sites Oz, O2, P2, A1, and A2 to maximize coverage of the primary visual cortex. The Oz electrode site was used for all analyses because this site displayed the FVEP-P2 with the largest amplitude for all study participants. Participants were instructed to remain still with their eyes closed and to press a hand-held button each time they saw the strobe flash. During the entirety of the experiment, a speaker delivered 90 dB white noise to mask the click of the strobe discharge.

Design

The FVEP-P2 latency was used as the dependent variable in a 2 × 6 mixed factorial design. The between-subjects factor possessed two levels: controls and MCIa patients, while the within-subjects factor possessed six levels: one single and five double stimulation (i.e., ISIs of 60, 70, 80, 100, and 120 ms) conditions. A single strobe flash was used in the single stimulation condition, while two successive strobe flashes were used in the double stimulation conditions. Each condition was composed of a block of 13.7 cm diameter, xenon strobe flashes of 5.5 lm s/ft² intensity. The duration of each strobe flash was 10 μs. One hundred strobe flashes in each condition were randomly presented (M = 1.5 s, range = 1.25–1.75 s) to prevent habituation. Each participant completed the same ABBA counterbalanced sequence: the six conditions being presented in a pre-set pseudo random order, a 15-min break, and then the same sequence presented in the reverse order.

Scan analyses and preprocessing of electroencephalography data

Electroencephalographic data were recorded with a sampling rate of 1000 Hz and bandpass filtered (0.1–35 Hz) to remove ambient electrical activity (e.g., 60 Hz ripple). An independent component analysis was performed to identify and remove EEG activity associated with eye blinks and other movements. Electroencephalographic data were visually inspected for artifacts and then epoched on each trial around the flash or pair of flashes (−200 to +500 ms). The 100 epochs in each condition were then averaged together to form separate VEPs for each EEG channel. To avoid over fitting of the data, a standardized semi-automatic algorithm was used on de-identified data to identify the FVEP-P2 associated with each flash. This procedure involved using the P2 latency to the single flash condition, identified as the maximum positivity between 100 and 300 ms after the flash, to create a time window that could be used to identify both P2s in the double flash conditions. The P2 associated with the first flash was identified as the maximum positivity within the time window that was centered on the previously calculated single flash latency (±100 ms). The second P2 was then identified as the first large positivity occurring after the P2 associated with the first flash and within the appropriate time window.

Results

Group differences

Table 1 summarizes the descriptive statistics for the MCIa and control groups. Expected differences were found on the Immediate and Delayed Memory subscales of the RBANS with no significant group differences on the other RBANS subscales. Age differed significantly between the groups, so it was added as a covariate for all between-groups analyses.

The main and interaction effects were calculated for the 2 × 6 mixed factorial analysis of variance using FVEP-P2 latency as the dependent variable. Mauchly’s Test of Sphericity was significant (p = 0.008), indicating a violation of the repeated measures sphericity assumption. Greenhouse–Geisser F-tests, which adjust the degrees of freedom to compensate for a violated sphericity assumption, were used for the within-subjects main effect of ISI, the Group × ISI interaction effect, and the simple main effect of Group at each level of ISI. Although the main and interaction effects were calculated, the hypotheses of the current investigation focused on group differences in each individual ISI condition. As such, simple main effects tests designed to assess the effects of Group at each level of ISI were employed.

The main effect of group was marginally significant with the MCIa group (M = 202.57, SD = 27.80) displaying longer P2 latencies than the control group (M = 163.92, SD = 29.99), F(1, 11) = 3.35, p = 0.101, partial η² = 0.27, d = 1.25. Neither the main effect of ISI nor the interaction effect of Group × ISI was significant.

The simple main effect of group was used to evaluate P2 latency differences separately for each ISI. Congruent with previous literature (Swanwick, et al., 1996), the P2 latency was significantly longer...
Discussion

The purpose of the present pilot investigation was to assess the clinical utility of a novel FVEP-P2 procedure for the diagnosis of MCIa. It was predicted that patients who were identified as having MCIa would exhibit longer FVEP-P2 latencies than a sample of healthy controls. Findings of the present investigation provide general support for this prediction and extend it by demonstrating high diagnostic accuracy for both the single and two of the double stimulation procedures.

Consistent with previous research (Swanwick, et al., 1996), individuals identified as being MCIa exhibited FVEP-P2 latencies that were longer than the FVEP-P2 latencies exhibited by healthy controls. Although the main effect was only marginally significant (p = 0.101), the large effect size (d = 1.25) suggests that the difference in luminance channel performance between those with MCIa and healthy controls may be clinically meaningful. Similar to the overall effect of group, the single flash condition also produced a large effect size (d = 2.47) that was statistically significant (p = 0.017), lending stronger support for the main hypothesis of this investigation. These results suggest that the cholinergic dysfunction apparent in AD is manifest in individuals identified as being MCIa, supporting the theory that MCIa may be an intermediate stage in the progression from normal aging to AD.

The double stimulation conditions produced results that partially support the other hypotheses. Contrary to what was predicted, no group differences were observed in P2 latency for the double flash conditions that immediately followed the refractory period (i.e., 60, 70, or 80 ms ISIs). The challenge of two stimuli presented with an ISI slightly longer than the refractory period (i.e., 50 ms) did not result in an increased difference in P2 latency delay. Instead, the two groups displayed more similar P2 latencies than were found in the single flash condition. This similarity did not hold true for all the double flash conditions, though, as significant group differences were found for the 100 (d = 1.60) and 120 ms (d = 1.86) double flash conditions. These results are particularly promising considering that other previously published

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in the single flash condition for MCIa patients (M = 199.22, SD = 13.06) than for controls (M = 159.01, SD = 17.59), F(1, 11) = 8.49, p = 0.017, partial η² = 0.49, d = 2.47. Significant group differences in P2 latency were not observed in the double stimulation conditions that had ISIs that were slightly longer than the refractory period of 50 ms (i.e., 60, 70, and 80 ms). However, the MCIa group exhibited significantly longer P2 latencies for the 100 ms, F(1, 11) = 5.36, p = 0.046, partial η² = 0.37, d = 1.60, and for the 120 ms double flash conditions, F(1, 11) = 5.98, p = 0.037, partial η² = 0.40, d = 1.86. Surprisingly, the control group had P2 latencies for the 100 (M = 154.74, SD = 32.16) and 120 ms (M = 152.11, SD = 18.43) double flash conditions that fell below their single flash latency.

The simple main effects tests of ISI at each level of Group were used to evaluate P2 latency differences across conditions separately for each group (Table 2). The MCIa group displayed strikingly similar P2 latencies across conditions that were not significantly different, indicating that no refractory effect was present—a finding that is consistent with a compromised cholinergic system. The control group demonstrated a clear refractory effect with P2 latency differences across conditions that were statistically significant, F(2.50, 17.48) = 4.12, p = 0.027, partial η² = 0.37. Compared with the single flash condition, the P2 latency delay increased for the relatively short ISI double stimulation conditions and then gradually returned to baseline as the ISIs became longer and more distal from the refractory threshold.

Discussion

The purpose of the present pilot investigation was to assess the clinical utility of a novel FVEP-P2 procedure for the diagnosis of MCIa. It was predicted that

Table 2 The simple main effects of group at each level of ISI on FVEP-P2 latency

<table>
<thead>
<tr>
<th>ISI</th>
<th>Controls (n = 8; females = 4)</th>
<th>MCIa (n = 5; females = 4)</th>
<th>F</th>
<th>p</th>
<th>Partial η²</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SD</td>
<td>M</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>164.54</td>
<td>31.02</td>
<td>200.62</td>
<td>8.49</td>
<td>0.017</td>
<td>0.49</td>
</tr>
<tr>
<td>70</td>
<td>167.61</td>
<td>42.36</td>
<td>202.14</td>
<td>5.53</td>
<td>0.056</td>
<td>0.17</td>
</tr>
<tr>
<td>80</td>
<td>164.37</td>
<td>38.35</td>
<td>201.26</td>
<td>4.04</td>
<td>0.06</td>
<td>0.36</td>
</tr>
<tr>
<td>100</td>
<td>154.74</td>
<td>32.16</td>
<td>208.14</td>
<td>1.74</td>
<td>0.16</td>
<td>0.29</td>
</tr>
<tr>
<td>120</td>
<td>152.11</td>
<td>18.43</td>
<td>204.04</td>
<td>3.98</td>
<td>0.046</td>
<td>0.40</td>
</tr>
<tr>
<td>Total</td>
<td>163.93</td>
<td>29.99</td>
<td>202.57</td>
<td>2.35</td>
<td>0.101</td>
<td>0.27</td>
</tr>
</tbody>
</table>

ISI, inter-stimulus interval; FVEP, flash visual evoked potential; MCIa, amnesic mild cognitive impairment.
investigations that have examined AD have reported smaller effect sizes (e.g., \( d = 0.68 \)) and lower sensitivity and specificity values (Coburn, et al., 2003).

The pattern of group differences observed across conditions may help shed some light on the status of the cholinergic system in individuals identified as having MCIa. The control group displayed a dynamic pattern of responses across ISI conditions that coincided with our predictions concerning the refractory effect, while the MCIa group did not. Compared with the single flash condition, the control group had a P2 latency increase in the double flash conditions with ISIs just longer than the refractory period that eventually returned to baseline in the longer ISI conditions. This pattern is a clear example of a refractory effect, in which responses that are abnormal for conditions more proximal to the refractory threshold return to normal in conditions more distal. The MCIa group did not display the same pattern of selective delays, as their P2 latencies were long and relatively constant across ISI conditions. Taken together, it appears that the challenge of the double stimulation procedure may have had very little effect on an already compromised cholinergic system, producing a pattern of latencies that may be indicative of the early stages of AD.

Limitations and future directions

There are several limitations to consider when interpreting the findings of this study. The most significant limitation was the small sample size, which

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**Figure 1** Grand average waveforms depicted as change in millivolts across time (ms) at site Oz. MCIa, amnesic mild cognitive impairment.
resulted in low statistical power. However, the present results are not only consistent with the findings of previous research and theory, but the effect sizes obtained in the present investigation were considerably greater than those observed in previous studies involving AD (Coburn, et al., 2003). Because effect size indicators are descriptive in nature, the observed effect sizes reported here suggest that meaningful differences in FVEP-P2 latencies are genuine and not an anomaly. As such, further investigation with larger sample sizes is needed to validate the present findings.

A second limitation involved the FVEP-P2 waveform identification. A semi-automated procedure was used to identify each P2 and to protect against experimenter bias. While this procedure produced numerical latency data, it did not detect the unpredicted amplitude differences that are apparent in the grand average waveforms (Figure 1). The low amplitude second P2 produced by the MCIa group in the double stimulation conditions may be indicative of a profound refractory effect that prolongs the refractory threshold and prevents the compromised visual system from responding to the second flash. Unfortunately, the magnitude of this refractory effect and its influence on P2 amplitude was unexpected, so the range of double stimulation ISI conditions tested in the present investigation was not wide enough to examine how long the refractory period extended and at what ISI a second P2 emerged and returned to a latency and amplitude similar to the single flash condition. Additional studies are needed to verify the present findings and explore the possibility that examining a large spread of ISIs could prove diagnostically useful in determining the health of the cholinergic system by measuring how severe a refractory effect is present and how long it takes the visual system to recover from the challenge of two rapidly presented flash stimuli.

Conclusions

The present study found significant differences in early visual processing between participants identified as having MCIa and healthy controls. Consistent with previous studies, group differences in FVEP-P2 latency were found in the single flash condition. Results from the double stimulation conditions revealed refractory effect differences between the MCIa and control groups, a dynamic that may represent a novel contribution to the literature on MCIa. These findings support the theory that MCIa is an intermediate stage between normal, healthy aging and the more severe pathology of AD.

Conflict of interest

None declared.

Key points

- Significant group differences in FVEP-P2 latency were observed in the single flash and 100ms and 120ms double stimulation conditions.
- The control group displayed a refractory effect such that P2 latencies were longer for double stimulation conditions with an ISI close to the refractory threshold of 50ms.
- The MCIa group had difficulty mounting a P2 response to the second flash in all double stimulation conditions, suggesting they have severe early visual processing deficit and extended refractory threshold.

References


