Research report

In vivo evidence for neuroplasticity in older adults

Fábio Henrique de Gobbi Porto\textsuperscript{a}, Anne Murphy Fox\textsuperscript{a}, Erich S. Tusch\textsuperscript{a}, Farzaneh Sorond\textsuperscript{b}, Abdul H. Mohammed\textsuperscript{c,d}, Kirk R. Daffner\textsuperscript{a,∗}

\textsuperscript{a} Laboratory of Healthy Cognitive Aging, Division of Cognitive and Behavioral Neurology and Center for Brain/Mind Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA
\textsuperscript{b} Division of Stroke and Cerebrovascular Disease, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA
\textsuperscript{c} Department of Psychology, Linnaeus University, Växjö, Sweden
\textsuperscript{d} Center for Alzheimer Research, Department of NVS, Karolinska Institutet, Huddinge, Sweden

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ABSTRACT

Neuroplasticity can be conceptualized as an intrinsic property of the brain that enables modification of function and structure in response to environmental demands. Neural plasticity is believed to serve as a critical mechanism underlying learning, memory, and other cognitive functions. Previous work investigating neuroplasticity has been done on hippocampal slices using high frequency stimulation. However, in vivo neuroplasticity in humans has been difficult to demonstrate. Recently, a long-term potentiation-like phenomenon, a form of neuroplastic change, was identified in young adults by differences in visual evoked potentials (VEPs) that were measured before and after tetanic visual stimulation (TVS). The current study investigated whether neuroplastic changes in the visual pathway can persist in older adults. Seventeen healthy subjects, 65 years and older, were recruited from the community. Subjects had a mean age of 77.4 years, a mean education of 17 years, and a mean MMSE of 29.1, and demonstrated normal performance on neuropsychological tests. 1 Hz checkerboard stimulation, presented randomly to the right or left visual hemifield, was followed by 2 min of 9 Hz stimulation (TVS) to one hemifield. After 2 min of rest, 1 Hz stimulation was repeated. Temporospatial principal component analysis was used to identify the N1b component of the VEPs, at lateral occipital locations, in response to 1 Hz stimulation pre- and post-TVS. Results showed that the amplitude of factors representing the early and late N1b component was substantially larger after tetanic stimulation. These findings indicate that high frequency visual stimulation can enhance the N1b in cognitively high functioning older adults, suggesting that neuroplastic changes in visual pathways can continue into late life. Future studies are needed to determine the extent to which this marker of neuroplasticity is sustained over a longer period of time, and is influenced by age, cognitive status, and neurodegenerative disease.

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1. Introduction

Neuroplasticity can be conceptualized as an intrinsic property of the brain that enables modification of function and structure in response to environmental demands, via the strengthening, weakening, pruning, or addition of synaptic connections, and by promoting neurogenesis (Pascual-Leone et al., 2011). There is presynaptically-mediated short-term plasticity lasting hundreds of milliseconds to a few minutes (e.g., posttetanic potentiation), and postsynaptically-mediated long-term plasticity (potentiation or depression), lasting minutes to months (Nicholls et al., 2011; Lüscher and Malenka, 2012; Regehr, 2012).

Posttetanic potentiation (PTP) is an example of a presynaptic form of short-term neuroplasticity that typically lasts 1–5 min (Catterall and Few, 2008; Regehr, 2012; Xu et al., 2007). PTP is driven by an augmented concentration of intracellular Ca\textsuperscript{2+} that is...
associated with increased probability of the release of neurotransmitters such as glutamate (Catterall and Febow, 2008; Fioravante and Regehr, 2011; Habets and Borst, 2006; Korogod et al., 2007; Xu et al., 2007). Its duration parallels the decay of intracellular Ca²⁺ (Nicholls et al., 2011). PTP plays several important regulatory roles in synaptic function and information processing (Regehr, 2012), and has been implicated as a synaptic mechanism underlying a number of short-term cognitive processes, such as working memory (Hansel and Matoto, 2013; Mongillo et al., 2008).

Long-term potentiation (LTP) is defined as a long-lasting enhancement in the efficacy of synaptic communication (Malenka and Nicoll, 1999; Lüscher and Malenka, 2012; Shapiro, 2001) that serves as a key cellular and biochemical mechanism related to memory formation (Cavus et al., 2012; Martin et al., 2000). LTP is triggered by modulation of ionotropic receptors such as N-methyl-D-aspartate receptor (NMDAR) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR), in the postsynaptic membrane. The most common excitatory neurotransmitter involved is glutamate. The early or induction phase of LTP is stimulus-dependent (e.g., contingent upon tetanic stimulation), and requires the depolarization of the presynaptic neurons and the presence of glutamate (Byth, 2014). This depolarization is associated with the removal of Mg²⁺ from the NMDAR, allowing calcium influx through the receptor, which triggers a complex intracellular cascade that leads to modifications of synaptic efficacy (Nicholls et al., 2011; Lüscher and Malenka, 2012). Due to activation of certain kinases and phosphorylation of targeted proteins, the plasticity becomes a long-lasting (i.e., minutes to months), stimulus-independent postsynaptic process known as LTP. Calcium works as a second messenger, altering the functioning of the postsynaptic neuron by increasing the sensitivity of AMPAR (via its phosphorylation) to glutamate, and by insertion of additional AMPARs in the postsynaptic membrane from a reserve pool (i.e., receptor trafficking). The influx of Ca²⁺ through NMDARs is believed to be a critical mechanism for the induction of LTP in the hippocampus (Bao et al., 1997; Bliss and Collingridge, 1993; Bliss and Lomo, 1973; Byth, 2014; Lüscher and Malenka, 2012; Malenka and Bear, 2004; Malenka and Nicoll, 1999; Maren et al., 1994). The exact time-frame of the transition period between presynaptic PTP and early LTP is uncertain (Byth, 2014).

External “artificial” high frequency stimulation has been shown to induce neuroplasticity in the form of PTP and LTP in hippocampal slices (Baez et al., 2013; Bliss and Collingridge, 1993; Habets and Borst, 2005, 2006; Korogod et al., 2007; Martin and Morris, 2002). Although the majority of studies on neuroplasticity have focused on excitatory synapses in the hippocampus, other areas of the mammalian brain, such as the visual system, likely share many of the excitatory synapse’s fundamental properties (Chen et al., 1996; Huang et al., 2014). Furthermore, although neuroplasticity is vital for understanding mechanisms underlying learning and memory, most research has investigated the phenomenon ex vivo—slices of hippocampal or cortical tissue from animals or humans. In vivo neuroplasticity has been demonstrated in animals using invasive techniques (Bliss and Gardner-Medwin, 1973; Bliss and Lomo, 1973). However, there have been few studies exploring this process in humans, using in vivo techniques. Recent reports suggest that an LTP-like phenomenon (LTP-lp)¹ can be demonstrated in vivo in young adults, using visual evoked potentials (VEPs) as the dependent variable (Cavus et al., 2012; Clapp et al., 2012, 2005; McNair et al., 2006; Ross et al., 2008; Teyler et al., 2005). Tetanic visual stimulation (TVS) at a frequency of 9 Hz induces an augmentation in the N1b component of the VEP, which has been shown by comparing the amplitude of the N1 before and after tetanic stimulation. In these studies, the augmented N1b response has been measured within 2 min of a TVS and has persisted for at least an hour, which was the duration of the experiments. In subsequent studies, TVS-induced LTP-lp has been shown to occur only if the stimuli used during tetanic presentation have the same physical properties (e.g., spatial frequency or orientation) as the visual stimuli presented pre- and post-tetanus (McNair et al., 2006; Ross et al., 2008). This kind of stimulus specificity is a core feature of LTP (Malenka and Nicoll, 1999; Lüscher and Malenka, 2012; Shapiro, 2001). A source modeling study using functional magnetic resonance imaging indicated that TVS-induced LTP-lp was most likely generated in the extrastriate cortex (Broadmann’s areas 18 and 19) (Clapp et al., 2005). Although ERP studies of young adults have varied in their use of principal component analysis (PCA) (Cavus et al., 2012), independent component analysis (ICA) (Teyler et al., 2005), and averaged waveforms analysis to identify and measure the N1b component (McNair et al., 2006; Ross et al., 2008), a robust potentiation of the N1b with TVS has been consistently observed. In summary, the use of VEPs appears to be a reliable method for non-invasively measuring TVS-induced LTP-lp in vivo, a surrogate marker of synaptic plasticity (Clapp et al., 2012).

An outstanding question involves whether TVS-induced neuroplastic changes continue into old age. This issue has important implications for understanding mechanisms underlying age-related differences in cognitive processes and synaptic plasticity (Malenka and Nicoll, 1999; Martin et al., 2000; Shapiro, 2001), as several predictable changes associated with normal aging may undermine the biological conditions in which neuroplasticity is sustained. The glutamatergic system is particularly susceptible to age-related disruption by oxidative, metabolic, and ionic stresses (Mattson and Magnus, 2006; Newcomer and Krystal, 2001), raising uncertainty about whether neuroplasticity would be observed in the aging brain (Burke and Barnes, 2006). Studies using transcranial magnetic stimulation (TMS), another model of in vivo neuroplasticity, have shown decreased motor cortex LTP-lp induced by paired associative stimulation in older adults, as compared to young adults (Fathi et al., 2010; Müller-Dahlhaus et al., 2008). The response appears to be more disrupted in older women than in older men (Teccio et al., 2008). In sum, the neurochemical and TMS data suggest that the aging brain may have decreased capacity for undergoing neuroplasticity. Of note, an abstract presented at the Society of Neuroscience (Tippett et al., 2011) reported ERP evidence of TVS-induced LTP-lp in some older subjects, lasting at least 30 min. Interestingly, subjects who demonstrated the LTP-like phenomenon had better scores on a familiarity-based recognition memory task. The aim of the current investigation was to use VEPs to determine if TVS-induced neuroplastic changes are present in cognitively normal older adults.

2. Methods and materials

2.1. Participants

Subjects 65 and older were recruited through community announcements in the Boston metropolitan area. All participants underwent an informed consent process approved by the Partners Human Research Committee. Inclusion criteria required subjects to be English-speaking, have 12 or more years of education, a Mini-Mental State Exam (MMSE) (Folstein et al., 1975) score ≥26, and

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¹ The phenomenon reported by other authors using in vivo models will be labeled throughout this report as “LTP-like phenomenon” rather than LTP. Despite previous data showing similarities between results using VEPs and hippocampal slices, without invasive recordings, it is not possible to be sure that the site of plasticity underlying the changes in VEPs is in the synapse. Therefore, we will use the term LTP-like phenomenon.
Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean (SD)</th>
<th>Variable</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>77.4 (6.1)</td>
<td>DS</td>
<td>61.1 (14.3)</td>
</tr>
<tr>
<td>Gender (F)</td>
<td>16 (34.1%)</td>
<td>FAS</td>
<td>43.6 (11.8)</td>
</tr>
<tr>
<td>Educ (yrs)</td>
<td>17.2 (8.5)</td>
<td>CF</td>
<td>42.8 (10.4)</td>
</tr>
<tr>
<td>MMSE</td>
<td>29.1 (0.8)</td>
<td>LM</td>
<td>28.1 (6.3)</td>
</tr>
<tr>
<td>AMNART</td>
<td>122.4 (3.5)</td>
<td>GDS</td>
<td>2.6 (2.3)</td>
</tr>
<tr>
<td>BNT</td>
<td>14.4 (1.1)</td>
<td>VA1</td>
<td>0.7 (0.2)</td>
</tr>
<tr>
<td>TMTA</td>
<td>41.8 (17.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMTB</td>
<td>91.8 (31.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AMNART, American National Adult Reading Test; BNT, Boston Naming Test; CF, category fluency (fruits, vegetables, and animals; words within each category/1 min); DS, digit symbol coding, WAIS-IV; Educ, education; F, female; FAS, phonemic fluency (with the letters “F,” “A,” and “S”; words beginning with each letter/1 min); GDS, Geriatric Depression Scale; LM, logical memory; TMT, trail making test (parts “A” and “B”); Wechsler Memory Scale-Third Edition; MMSE, Mini-Mental State Examination; SD, standard deviation; VA, visual acuity; yrs, years.

1 Number of cases and percentage of total.

2 Binocular visual acuity was measured in all subjects with the Snellen 10 ft model wall chart and recorded as a decimal representation of 20/x, such that 20/20 = 1.0.

an estimated intelligence quotient (IQ), based on the American National Adult Reading Test (AMNART) (Ryan and Paolo, 1992) > 90. Subjects were excluded if they had a history of clinically significant CNS or medical disease, major psychiatric disorders based on DSM-IV (American Psychiatric Association, 1994) criteria, hearing or visual impairment that would prevent them from being able to follow verbal instructions or complete neuropsychological testing. Geriatric Depression Scale (GDS) (Yesavage et al., 1983) score ≥ 10, or focal abnormalities on neurological examination consistent with a CNS lesion.

Seventeen older adults were included in this study. Table 1 summarizes the pertinent demographic and neuropsychological data on the subjects. Of note, they had completed a mean of 17 (2.8) years of education and had an estimated IQ of 122.4 (3.5).

2.2. Experimental procedures

Following the methods used by Teyler et al. (2005), and in accordance with the International Society for Clinical Electrophysiology of Vision recommendations (Odom et al., 2010), VEPs were elicited by high contrast black and white checkerboard stimuli with 0.4 degrees of arc and boxes measuring 1 cm on each side. Participants were instructed to fixate on a red cross on the center of the screen during all data recordings. The viewing distance was approximately 154 cm. Checkerboards were presented randomly to the right or left visual hemi-field for a total duration of 2 min (50% on each side). The stimulus duration was 33 ms and the inter-stimulus interval ranged from 917 to 1017 ms (average presentation rate of ~1 Hz).

After subjects had rested for 15 s with their eyes open, 2 min of TVS was presented to one hemi-field (counterbalanced across subjects between the right and left hemi-fields). TVS consisted of a checkerboard with the same color, contrast, and luminance of the previous 1 Hz stimulus, but with a frequency of 9 Hz (which is below perceptual fusion rate). Following the TVS, participants were instructed to remain at rest, with their eyes closed for 2 min, in order to avoid TVS-induced “after-images” in the subsequent recording. After this rest period, checkerboard stimuli were presented at 1 Hz for 2 min, following the same procedure used during the baseline recording.

2.3. ERP recordings

An ActiveTwo electrode cap (Behavioral Brain Sciences Center, Birmingham, UK) was used to hold to a full array of 128 Ag–AgCl BioSemi (Amsterdam, The Netherlands) “active” electrodes to the scalp, at locations determined by a pre-configured montage. Electrodes were arranged in equidistant concentric circles from the International 10–20 system position Cz. In addition to the 128 electrodes on the scalp, 6 mini bio-potential electrodes were placed, over the left and right mastoid, beneath each eye, and next to the outer canthi of the eyes to capture eye blinks and vertical and horizontal eye movements. EEG activity was digitized at a sampling rate of 512 Hz.

2.4. Data analysis

EEG data were analyzed using ERPLAB (www.erpinfo.org/erplab) (Lopez-Calderon and Luck, 2014) and EEGLAB (http://sccn.ucsd.edu/eeeglab) toolboxes that operate within the MATLAB framework (Delorme and Makeig, 2004). Raw EEG data were resampled to 256 Hz and referenced off-line to the algebraic average of the right and left mastoids. EEG signals were filtered using an IIR filter with a bandwidth of 0.03–40 Hz (12 dB/octave roll-off). Eye artifacts were removed through an independent component analysis. Individual bad channels were corrected with the EEGLAB interpolation function. Epochs were discarded from the analyses if they contained baseline drift or movement artifacts greater than ±90 μV. Data were analyzed as a function of electrode sites contralateral or ipsilateral to tetanic stimulation. In the figures, the electrophysiological activity contralateral to tetanic stimulation is displayed on the right side of the scalp topographic maps.

2.5. PCA analyses

Following the recommendations of Dien et al. (2007) and Dien (2010a), a two-step temporospatial procedure (temporal PCA with Promax rotation followed by a spatial PCA with Infomax rotation, the latter of which is equivalent to independent component analysis) was conducted on all subjects’ individual ERP averages, at all 134 electrode sites, using the ERP PCA toolkit 2.39 (Dien et al., 2007; Dien, 2010b). PCA is a data-driven method that decomposes ERP waveforms into their underlying components and is particularly useful in parsing spatially and temporally overlapping components. Following an approach shown to provide increased sensitivity for identifying differences between conditions (Cohen, 2014), VEPs that were recorded before and after TVS were analyzed in separate PCAs. The time window was limited to 0–350 ms, with a 200 ms baseline. A parallel test was used to restrict the number of factors generated for each PCA (Dien, 2012). Examination of the latency and topography of the PCA output led to the identification of factors of interest. Factor scores (amplitudes) were submitted to statistical analysis using paired t-tests.

3. Results

Based on visual inspection of the timing and topography of the factors, two were of particular interest to the goals of this study, as they represented N1 subcomponents. One factor peaked around 132 ms (128 ms for pre-VEPs and 136 ms for post-VEPs) in the lateral occipital region contralateral to the side of tetanic stimulation, which was labeled as the early N1b component. Another factor peaked at 183 ms (for both pre- and post-VEPs) in the lateral occipital region (just lateral to the location of the early N1b peak), which was labeled as the late N1b component.

Fig. 1 illustrates the temporospatial factors representing the early and late N1b components pre- and post-tetanus. The amplitude of the factors representing the early and late N1b components was larger (more negative) after TVS than before (for the early N1b component, post: −5.02 (5.6) μV; pre: −1.97 (5.8) μV; difference: −3.04 (2.2) μV, p < 0.001; for the late N1b component, post: −3.76 μV, p = 0.035).
(2.3) μV; pre: −2.48 (2.0) μV; difference: −1.28 (1.3) μV, p = 0.001). Of note, there was no correlation between the early and late N1b values for the post-TVS – pre-TVS amplitude (p = 0.5).

4. Discussion

To the best of our knowledge, this is the first full-length published report demonstrating in vivo neuroplastic changes in older adults using VEPs. Our results show a reliable increase in the amplitudes of both early and late N1b components after TVS in cognitively normal older adults, an observation consistent with previous evidence of in vivo LTP-lp in older adults (Tippett et al., 2011). Studies of young adults using VEPs to investigate LTP-lp have relied on different methods for identifying relevant components (Cavus et al., 2012; McNair et al., 2006; Ross et al., 2008; Teyler et al., 2005). Most have employed single step ICA or PCA, or measurements based on the amplitude peaking between the N1 and P2 waves of the averaged ERP data. Despite some methodological differences, results in young adults have been relatively consistent across studies, demonstrating TVS-induced augmentation of the N1b component peaking between 100 and 150 ms (Cavus et al., 2012) or between 150 and 200 ms (McNair et al., 2006; Ross et al., 2008). The current study used a two-step procedure, with temporal PCA (with Promax rotation) followed by spatial PCA (with Infomax rotation), to identify and measure components of interest. This approach was used because it has been shown to produce results most consistent with simulated data sets (Dien et al., 2007; Dien, 2010b). TVS-induced neuroplasticity was demonstrated in old adults for two components, an early and late N1b. Of note, there was no correlation between the magnitude of TVS-induced augmentation of these two components, indicating that they do not measure identical underlying operations and are probably derived from distinct neural sources.

Our data were collected 2 min after the TVS, which is a period of time most consistent with PTP. There is no consensus regarding the exact temporal border between PTP and early LTP, and there is probably a period of overlap between the two forms of neuroplasticity when a tetanic stimulation is the inducing stimulus (Byth, 2014). The methodology used in the current investigation is similar to that employed in previous studies, which have demonstrated that neuroplastic changes measured after two minutes, consistent with PTP, continued to last at least 30 min in young (Cavus et al., 2012; McNair et al., 2006; Ross et al., 2008; Teyler et al., 2005) and older adults (Tippett et al., 2011), consistent with LTP-lp. Additional research is necessary to confirm whether the TVS-induced VEP potentiation in older adults observed in our study is sustained for a duration that is consistent with LTP-lp. It is critical to note that both short-term and long-term plasticity have important
implications for aging and cognition (Cavus et al., 2012; Hansel and Mato, 2013; Martin et al., 2000; Mongillo et al., 2008). Thus, the results of studies like the current one would be meaningful, whether they reflect PTP, LTP, or both. The methodology used may serve as a promising, non-invasive tool to measure neuroplasticity and study the brain’s aging process.

Our results are of particular interest, given reports of age-related changes in the glutamatergic system that may undermine conditions needed to facilitate neuroplasticity. The glutamatergic system appears to be especially susceptible to age-associated disruption by metabolic, oxidative, and ionic stresses (Mattson and Magnus, 2006; Newcomer and Krystal, 2001). Notably, the results of a study using an animal model with invasive in vivo VEP recordings demonstrated that an NMDA antagonist completely abolished LTP, suggesting that the glutamatergic system needs to be properly functioning to allow for neuroplasticity (Kang and Vaucher, 2009). Research in humans also has shown an age-related decrease in NMDAR function, which has been associated with declines in memory and learning (Newcomer and Krystal, 2001; Newcomer et al., 2000). Given this age-related vulnerability, it was unclear whether VEP augmentation would be observed. Our results demonstrate that some cognitively normal older adults have the capability to exhibit TVS-induced neuroplastic changes, thus providing evidence that their visual pathways are still able to undergo neuroplasticity. Furthermore, we demonstrated TVS-induced neuroplastic changes in older women (the majority of our subjects), a group that was shown to be particularly unresponsive to attempts to induce LTP-Ip in a TMS study (Tecchio et al., 2008).

This study has a few limitations. Our sample size was relatively small and not representative of the aging population. Participants were well-educated, highly intelligent, and predominantly female. Thus, the extent to which our results are generalizable remains unclear. Although the study demonstrated that neuroplasticity can persist into late adulthood (mean age of 77), and is supported by previous research on older individuals (Tippett et al., 2011), it remains to be determined if this phenomenon is a common feature of the aging human brain, or if TVS-induced VEP augmentation is limited to older adults with high cognitive reserve (Barulli and Stern, 2013). A follow-up investigation with a much larger, more heterogeneous sample is needed to address this issue. The study also did not include a comparison group of young adults. Future studies should determine if there are age-related differences in the magnitude of TVS-induced neuroplasticity. To determine whether TVS can induce neuroplastic changes in older adults consistent with LTP-Ip, it is essential to investigate if the augmentation in N1 amplitude is sustained over a duration of more than a few minutes and depends on stimulus specificity. Moreover, it would be informative to examine the links between TVS-induced electrophysiological changes, performance on visually mediated tasks, and biochemical and imaging markers of synaptic function. Finally, future studies are also needed to determine whether this presumed marker of neural plasticity is absent in neurodegenerative diseases.

5. Conclusion

Neuroplastic changes can be induced by TVS in cognitively normal older adults, at least in individuals who exhibit “successful” aging. Additional research is needed to clarify if TVS-induced neuroplastic changes are also present in a more heterogeneous older adult sample and to compare its magnitude across different age groups. Despite some unanswered questions, this non-invasive method for measuring neuroplasticity appears to be a promising tool to study the brain’s aging process.

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