Age-related changes in the relationship between visual evoked potentials and visually evoked cerebral blood flow velocity responses

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Summary

We evaluated neurovascular coupling during normal aging using visual evoked potentials (VEPs) and visually evoked cerebral blood flow velocity responses (VEFRs). The recordings were made from a group of healthy younger and older subjects. The stimulus was a black and white checkerboard with visual contrasts of 1%, 10% and 100%. The VEFRs were measured in the posterior cerebral artery using transcranial Doppler simultaneously with VEPs from occipital leads. A significant increase in the VEFRs and VEPs in response to graded visual contrasts (p<0.01) was found in both groups. Linear regression analysis showed a significant positive association between the VEPs and the VEFRs in the younger (r=0.66, p<0.01) and older subjects (r=0.74, p<0.01). We also found significant differences in neurovascular coupling index (VEFR/VEP) between both groups at each visual contrast (p<0.01). We conclude that simultaneous recording of VEFRs and VEPs at graded visual contrasts indicated attenuated neurovascular coupling in older subjects.

KEY WORDS: neurovascular coupling, transcranial Doppler, visual evoked potentials, visually evoked cerebral blood flow velocity responses, visual contrast.

Introduction

A number of functional imaging studies, most notably using positron emission tomography (PET) and functional magnetic resonance imaging (fMRI), both techniques based on the vascular response to brain activity, have sought to identify age-related changes in the neural substrates of cognitive processes. In addition, it has been shown that neurovascular coupling may play an important role in the pathogenesis of age-related neurological disorders such as vascular and neurodegenerative dementia (1). It has also been shown that transcranial Doppler sonography (TCD) measures cerebral perfusion changes related to neuronal activation in a manner similar to fMRI (2). Recent fMRI studies have demonstrated a reduction of cerebral activation in older subjects, which may be associated with age-related changes in the mechanism linking neuronal activity to vascular changes (3). A TCD study has observed an age-dependent decline of visually evoked cerebral blood flow velocity responses (VEFRs) (4). These data suggest that attenuated vascular responses during aging may be attributed to decreased neurovascular coupling activity.

To explore the relationship between neuronal activation and vascular responses in animals and humans, researchers have compared the amplitudes of stimulus-evoked potentials detected by fMRI, by near infrared spectroscopy, and by laser Doppler signals (5-7). One study demonstrated that TCD sensitivity is sufficient to detect VEFRs in the territory of the posterior cerebral artery (8). In the present study, noninvasive methods were used to examine the effect of aging on neurovascular coupling. To this end, we simultaneously recorded visual evoked potentials (VEPs) and VEFRs in two different age groups.

Materials and methods

Subjects and procedure

The recordings were made from 40 healthy volunteers aged 48.7±17.4 years (range: 22 to 78 years) of both sexes (21 women and 19 men). Neither the clinical and neurological examination nor the ultrasound examination of cerebral and precerebral arteries showed any hemodynamically significant stenosis. CT scans were performed in the subjects older than 55 years of age, and did not show any significant changes. We also administered a mini-mental test, which was normal in all the subjects. Visual acuity was investigated using Snellen cards; subjects with refractive errors used their normal prescription lenses during the recordings. All the subjects were asked not to drink caffeine-containing beverages and to refrain from smoking on the test day. Volunteers on any sort of medication were excluded. The study had been approved by the Medical Ethics Committee of the Republic of Slovenia. Informed consent was obtained from each volunteer.

The experiments were performed in a darkened, quiet room and always at the same time of day. Before the actual testing, the research protocol was explained to the
subjects, who were also asked to breathe regularly during the experiment. They were seated comfortably one meter from a computer screen which subtended 22 degrees of the subject's visual angle, and fixed their eyes on a small spot of red light in the center of the screen.

**Stimulus**

The visual stimulus was presented on the computer screen. It consisted of white and black checks arranged in a checkerboard pattern with a spatial frequency of 1.6 cycles per degree. The mean luminance of the visual stimulus was 28 cd/m². The luminance (L) of the checks was changed in order to obtain visual contrasts of 100%, 10% and 1%. Visual contrast (C) was defined according to the formula: $C = (L_{white} - L_{black}) / (L_{white} + L_{black})$. The mean luminance of the checkerboard at different visual contrasts remained unchanged.

**VEFR recording**

A multimodal recording of arterial pressure, end-tidal carbon dioxide (Et-CO₂) and arterial velocity was performed. Arterial pressure was continuously monitored using a blood pressure monitor (Colin 7000, Komaki City, Japan), Et-CO₂ was monitored using the Capnodig infrared capnograph (Draeger, Lübeck, Germany), and heart rate was determined by processing of Doppler signal using TCD8 commercial software. Arterial velocity was recorded in the left middle cerebral artery (MCA) and in the right posterior cerebral artery (PCA) through temporal acoustic windows by means of Multi-Dop X4 (DWL, Sipplingen, Germany), using 2 MHz transducers. The vessels were identified according to criteria described elsewhere (9). To confirm the localization of the vessels, we considered anatomic landmarks, direction of the flow, and compression maneuvers. The criterion for successful PCA insonation was a clear-cut flow velocity increase during the period when the subjects had their eyes open compared with the period when their eyes were closed. The P2 segment of the PCA was always the one insonated since it proved to have a higher visual response than the P1 segment (4). The MCA and the PCA were insonated at typical depths of 54 mm and 64 mm respectively. We tried to maintain this 10-mm depth difference between the MCA and the PCA.

**VEP recording**

The cerebral evoked activity was recorded from the scalp by means of silver-silver chloride cup electrodes (10 mm diameter) fixed using contact paste. The resistance was kept constantly below 5kΩ. The electrodes were placed according to the recommendations of the International Society for Clinical Electrophysiology of Vision (10). Three active electrodes were used, i.e., the electrode designated Oz, placed 10% of the inion-nasion distance above the inion, and the electrodes designated O1 and O2, placed 10% of the head circumference laterally to the left and right respectively. The reference electrode, designated Fz, was placed 20% of the inion-nasion distance frontally to the vertex. The VEP activity was fed to an amplifier system with a linear frequency response from 1 to 250 Hz, and displayed on an oscilloscope for continual observation. The analysis time was 600 msec, and the sensitivity 10 µV per division. The signal was subsequently conveyed to a computer system, which also served as an on-line averager. Five hundred responses were commonly averaged and presented on the oscilloscope screen before being stored on disc.

**Stimulus paradigm**

The experimental session consisted of stimulus eyes-open and stimulus eyes-closed periods. The stimulus eyes-open period lasted 70 seconds. During that time we presented the visual stimuli, i.e., the alternation of the checkerboard pattern with a diffuse white stimulus of equal mean luminance. The checkerboard pattern appeared for 200 msec and the diffuse white stimulus for 500 msec. The stimulus frequency was 1.4 Hz. Therefore, precisely 100 VEPs were obtained during the stimulus eyes-open period. The stimulus eyes-open period was repeated five times in a row at each visual contrast in order to obtain approximately 500 VEPs for each visual contrast. The stimulus eyes-closed period lasted 30 seconds. The order of the stimulus conditions was randomly varied for each subject.

**Evaluation of data on multimodal recording**

The multimodal recording (Fig. 1) included the baseline period before the start of stimulation, and the stimulation
periods. We determined the mean amplitudes of the blood flow velocities in the MCA (vMCA), in the PCA (vPCA), as well as the mean amplitudes of arterial pressure (MAP), heart rate (HR) and Et-CO₂, for the baseline condition (before the stimulation with eyes closed) as well as for each stimulus eyes-open period at 1%, 10% and 100% visual contrasts. The mean amplitudes were calculated by means of TCD8 software, using a commercial algorithm according to the formula:

\[ A_m = \frac{\int v \, dt}{t_0 - t_1} \]

in which \( A_m \) represents the mean amplitude of the variables (v) included in the measurements. The VEFRs were defined as the differences between the \( v_{PCA} \) in baseline condition and the \( v_{PCA} \) in the stimulus eyes-open periods at 1%, 10% and 100% visual contrasts. On the basis of the VEFRs, \( \delta_{vMCA} \), \( \delta_{MAP} \), \( \delta_{HR} \) and \( \delta_{Et-CO₂} \) were defined as the differences between the \( v_{MCA} \), MAP, HR and Et-CO₂ in baseline condition, and the \( v_{MCA} \), MAP, HR and Et-CO₂ in the stimulus eyes-open periods at 1%, 10% and 100% visual contrasts. We then calculated the mean values of five successive VEFRs, \( \delta_{vMCA} \), \( \delta_{MAP} \), \( \delta_{HR} \) and \( \delta_{Et-CO₂} \).

VEP data evaluation

The averaged responses of the VEPs, as measured from the active electrode at O1, were analyzed off-line on a computer system by measuring the amplitudes in the interval of interest. We had previously designed a software which enabled us to calculate the mean absolute amplitude \( |A| \) of the VEP according to the formula:

\[ |A| = \frac{\sum |A|}{n} \]

in which A is the amplitude of the sample and n the number of samples during the chosen period. The amplitudes of the samples were measured from baseline. The sampling frequency was 1.67 samples/msec. The mean absolute amplitude was calculated for the interval from 50 to 200 msec, the interval in which early onset VEP response typically occurs.

Statistical methods

ANOVA for repeated measures was used to analyze differences between more than two successive measurement data in the same group. If a model showed significant variances, a paired t-test with Bonferroni correction was used to locate the point of significance. In order to evaluate relationships, a linear regression model was applied. All the statistical analyses were performed using SPSS statistical software. The differences were considered significant when p<0.05. In order to analyze the difference between two slopes of the regression curves, a t-test was applied (11).

Results

The subjects were divided according to age into two groups. The first comprised 26 subjects aged under 55 years and the second 14 subjects aged 55 or over. The mean age of the younger group was 37.5±9.4 years, and of the older group 69.5±5.9 years. In all the subjects, the velocity in the PCA increased during the stimulation at the different visual contrasts. Figure 1 shows a representative multimodal recording of the \( v_{MCA} \), \( v_{PCA} \), MAP, HR and Et-CO₂ in a 27-year-old man (lower) and in a 75-year-old man (upper). The \( v_{PCA} \) of both subjects showed responses to visual contrast, while the other variables did not show specific changes. We observed that responses in the \( v_{PCA} \) began immediately after the visual stimulation and showed no adaptation. They were highest at 100% visual contrast and lowest at 1% visual contrast. They also showed good reproducibility. The responses of the older subject were substantially smaller than those of the younger subject. Figure 2 shows VEPs in response to three different visual contrasts from three occipital leads (O1, O2 and Oz).}

**Figure 2** - Example of visual evoked potential (VEP) recordings at three different visual contrasts in the same two subjects as in Figure 1 (older left, younger right) from occipital leads (O1, O2, Oz).

We analyzed changes in the VEFRs at 1%, 10% and 100% visual contrast in both groups. In both the younger and the older subjects, the ANOVA for repeated measures showed significant differences between successive measurement data for the VEPs and the VEFRs (p<0.01). There were no significant differences between the VEPs of the older and the younger subjects (p=0.15) (Fig. 3, see over). However, we found significant differences between the VEFRs of the two groups, those of the younger subjects being significantly higher than those of the older ones (139% higher at 100% visual contrast).
contrast, 119% higher at 10% visual contrast and 129% higher at 1% visual contrast, p<0.01) (Fig. 4).

The other variables, i.e., δMCA, δMAP, δEt-CO2 and δHR, did not show any significant differences either in the older subjects (p=0.21, p=0.32, p=0.48 and p=0.37 respectively) or in the younger subjects (p=0.23, p=0.45, p=0.28 and p=0.34 respectively), within or between the two groups (p=0.54, p=0.42, p=0.38 and p=0.67 respectively).

We tested the relationships between the VEPs and the VEFRs in the younger as well as in the older subjects (Fig. 5). The linear regression showed a significant positive association between the VEPs and the VEFRs in the younger (r=0.66, p<0.01) and in the older subjects (r=0.74, p<0.01). The ratio VEFR/VEP was introduced as a neurovascular coupling index (Fig. 6). We found significant differences within (p<0.05) and between both groups at each visual contrast (p<0.01).

Figure 3 - The dependence of visual evoked potentials (VEPs) on visual contrasts in younger (solid line) and older (dotted line) subjects. The plot represents VEP means and standard deviations at different visual contrasts.

Figure 4 - The dependence of visually evoked cerebral blood flow velocity responses (VEFRs) at different visual contrasts in younger (solid line) and older (dotted line) subjects. The plot represents VEFR means and standard deviations at different visual contrasts.

Figure 5 - The scatter plot of visual evoked potential (VEP) amplitudes versus visually evoked cerebral blood flow velocity response (VEFR) amplitudes. Linear regression line was fitted to the experimental data of younger (dashed line) and older (solid line) subjects. Linear regression coefficient for younger subjects was b=0.54 and significance p<0.01. Linear regression coefficient for older subjects was b=0.40 and significance p<0.01. The difference between regression coefficients of older and younger subjects was significant (p<0.01). The younger group comprised twenty-six and the older group fourteen healthy subjects. Three observations were made for each subject at each visual contrast.

Figure 6 - The dependence of neurovascular coupling index (VEFR/VEP) on visual contrasts in younger (solid line) and older (dotted line) subjects. The plot represents neurovascular coupling index means and standard deviations at three different visual contrasts.
Discussion

Our study examined simultaneously the effects of visual contrast on VEPs and VEFRs in older and younger subjects. We found that higher visual contrasts produced higher VEFRs in all subjects. The increase of regional cerebral blood flow in the visual cortex due to metabolic demand after appropriate stimulation is well established and has been demonstrated by various functional imaging studies (2). Although arterial velocity cannot be used as an indicator of cerebral blood flow, changes in blood flow velocity have been found to correlate reliably with changes in cerebral blood flow, as long as the vessel diameter and the perfusion territory remain constant (12). It is well known that an increase in visual contrast is followed by an increase in neuronal activity. The visual system neurons, including those of the striate and extrastriate cortex, respond to visual contrasts (13). Indeed, the neurons in V1 increase their firing rates in response to increases in stimulus contrast, regardless of their selectivity (14). It is widely accepted that fMRI monitors regional cerebral blood flow. Our results, like those of fMRI studies, imply a direct relationship between VEFR response and neuronal activity.

We found that in the older group the vascular responses to visual stimulations were lower at all three visual contrasts compared to those of the younger group. This finding is in agreement with MRI studies, which showed an attenuation of the BOLD signal in older subjects (3), as well as with TCD studies of evoked flow (15). Age-related changes have been reported in the cerebral vasculature, including a thickening of the vascular basement membrane and a thinning of the endothelium (16). In addition, atherosclerotic changes in cerebrovascular ultrastructure during aging could reduce the dynamic range of vascular reactivity in the affected area. Less well established are the effects of aging upon cerebral metabolism. Some authors have reported age-related declines, whereas others have not (17). In addition, some studies have suggested that age-related changes in resting brain metabolism may differ across brain regions with significant changes occurring in the frontal cortex, and with little change reported in the visual cortex (18). Nevertheless, our study showed significant reduction of metabolic evoked flow in the visual cortex in older subjects. The changes in the vascular response to the neuronal activity that occur with age could well be the result of altered neurovascular coupling.

In order to clarify the influence of neurovascular coupling on vascular responses we simultaneously monitored VEPs and VEFRs. We found higher VEP amplitudes in response to increased visual contrast in both groups. Similar results have been obtained by other studies, which have explored the effect of visual contrast on VEP amplitude (19). The generator site is believed to be the peristriate and striate occipital cortex in the perfusion territory of the PCA. Albeit insignificant, we noted a reduction of VEP amplitudes. Physiological studies have revealed age-related changes in evoked potentials. Some studies report age-related declines in evoked potential amplitudes (20), others do not (21). Morphological studies have reported anatomical changes in the brain, which include sulcal widening, increased ventricular size, and loss of synapses (22). However, these structural changes may not be concomitant with the changes in neuronal density occurring in the course of normal aging. While early studies advanced the idea that the normal aging process is accompanied by neuronal loss, even in the absence of pathological states, recent studies using advanced sampling and stereological techniques have shown negligible neuronal loss (23). These studies have suggested that the functional decline observed in normal aging is not due to changes in neuronal density.

We found a linear relationship between VEPs and VEFRs in younger as well as in older subjects. Our major finding was that the neurovascular coupling index was significantly lower in the older subjects compared to the younger ones at each visual contrast. This finding suggested a diminished function of neurovascular coupling in older subjects. Although studies have explored the relationship between evoked potentials and vascular responses detected by fMRI (24) and near infrared spectroscopy (7), none of them has studied the effect of aging on this relationship. An indirect study using TCD with control system analysis has suggested that the neurovascular coupling mechanism is unaffected by moderate aging as estimated by Doppler parameters (25). However, in our study, as in other TCD studies that have also reported diminished responses in older subjects, the age of the subjects investigated was substantially greater (15).

We conclude that the simultaneous recording of VEFRs and VEPs in response to graded visual contrasts indicates a diminished activity of neurovascular coupling in older subjects and this has to be taken into consideration when interpreting the results of functional neuroimaging studies. The aging process may affect neurovascular coupling itself.

References

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