NEUROVASCULAR COUPLING DURING AGEING AND IN MIGRANEURS

ŽIVČNOŽILNA SKLOPITEV PRI STAREJŠIH IN BOLNIKIH Z MIGRENO

Marjan Zaletel

Department of Neurology, University Medical Centre Ljubljana, Zaloska c. 7, 1525 Ljubljana, Slovenia

Abstract

A noninvasive assessment of neurovascular coupling using visually evoked cerebral blood flow velocity responses (VEFR) and visual evoked potentials (VEP), during normal ageing and in migraneurs, would be of great importance for interpretation of functional neuroimaging methods. According to the recent findings neurovascular coupling could be altered in older subjects and in migraneurs.

The records were made from a group of healthy younger (37.5±9.4 years; 20 subjects) and older subjects (69.5±5.9 years; 20 subjects) as well as from patients with migraine (36.6 ± 10.4 years) interictally. The stimulus was a black-and-white checkerboard with visual contrasts of 1 %, 10 % and 100 %. The VEFR were measured in the posterior cerebral artery using transcranial Doppler (TCD), and the VEP were recoded from occipital leads. To test the relationship between the VEFR, the VEP and the visual contrast, a linear regression analysis was performed.

A significant increase of the VEFR and the VEP to graded visual contrasts ($p < 0.01$) was found both in the younger and older subjects. The linear regression showed a significant positive association between the VEP in the VEFR ($r = 0.66$, $p < 0.01$) of the younger and older subjects ($r = 0.74$, $p < 0.01$). The regression coefficient of the younger subjects was significantly higher ($b = 0.54$; $SE = 0.07$) than that of the older ones ($b = 0.40$; $SE = 0.05$) ($p < 0.01$). Also, we found an increase of VEFR and VEP within group of migraneurs ($p < 0.01$). VEFR were significantly higher in migraneurs ($p < 0.01$) compared to controls, while VEP did not significantly differ between the groups ($p > 0.05$). The regression showed a significant association between VEP and VEFR migraneurs ($r = 0.63$, $p < 0.01$). The regression coefficient of migraneurs ($b = 0.88$, $SE = 0.08$) was significantly higher than of healthy subjects ($b = 0.55$, $SE = 0.07$) ($p = 0.04$).

We concluded that a simultaneous recording of VEFR and VEP at graded visual contrasts indicates diminished neurovascular coupling in older subjects and it is increased in migraneurs interictally.

Key words

transcranial Doppler; visual evoked potentials; visually evoked cerebral blood flow velocity responses; migraine; ageing

Izvleček

Neinvazivna analiza živčnožilne sklopitve z merjenjem evociranih možganskih odgovorov krvnega pretoka (VEOP) in vidnih evociranih potencialov (VEP) bi lahko bila pomembna zaradi razlage rezultatov funkcijskih slikovnih metodv zvezi s staranjem in pri bolnikih z migreno. V skladu s dosedanjimi spoznanji je živčnožilna sklopitev lahko spremenjena pri starejših in bolnikih z migreno.

V raziskavi je sodelovalo20 zdravih mlajših (37,5 ± 9,4 leta) in 20 starejših preiskovancev (69,5 ± 5,9 leta) ter 30 bolnikov z migreno (36,6 ± 10,4 leta). Za svetlobni dražljaj smo uporabili šahovnico, na kateri smo spreminjali vidni kontrast. Uporabili smo 1-, 10- in 100-odstotni vidni kontrast. VEOP smo merili v zadnji možganski arteriji s transkranialnim Dopplerjem (TCD). VEP smo snemali v zatiljnih odvodih.

VEOP in VEP so se pomembno povečali po draženju z različnim kontrastom ($p < 0,01$). Linearna regresija je pokazala statistično pomembno povezanost med VEOP in VEP pri starejših ($r = 0,66$, $p < 0,01$) in mlajših preiskovancih ($r = 0,74$, $p < 0,01$). Regresijski

Correspondence / Dopisovanje:

Marjan Zaletel, MD, MSc, University Medical Centre Ljubljana, Department of Neurology, Zaloska c. 7, SI-1525 Ljubljana, Slovenia, phone: +386-41 761 288; Fax: +386-1-522 22 08, e-mail: marjan.zaletel@kclj.si

koeficient je bil pomembno večji pri mlajših (b = 0,54; SE = 0,07) kot pri starejših preiskovancih (b = 0,40; SE = 0,05) (p < 0,01). Ugotovili smo tudi pomembno povečanje VEOP in VEP v skupini bolnikov z migreno (p < 0,01). VEOP so bili pri bolnikih z migreno pomembno večji (p < 0,01), VEP pa se med skupinama niso pomembno razlikovali (p > 0,05). Regresija je pokazala pomembno povezanost med VEP in VEOP pri bolnikih z migreno (r = 0,63, p < 0,01). Regresijski koeficient je bil pri bolnikih z migreno (b = 0,88, SE = 0,08) pomembno večji kot kot pri zdravih preiskovancih (b = 0,55, SE = 0,07) (p = 0,04).
Zaključili smo, da sočasno snemanje VEOP in VEP pri spreminjajočem se vidnem kontrastu kaže na zmanjšano živčnožilno sklopitev pri starejših bolnikih in povečano pri bolnikih z migreno v času brez migrenskega napada.

Ključne besede *transkranialni dopler; vidni evocirani potenciali; evocirani možganski odgovori krvnega pretoka; migrena; staranje*

Introduction

Neuronal activity, cerebral metabolism and regional cerebral blood flow are tightly coupled. The term neurovascular coupling denotes the relationship between neuronal activity and regional cerebral blood flow. It enables the maintenance of regional cerebral blood flow as well as its adaptation to regional neuronal activity and metabolism; it is thus essential for the normal functioning of the brain. The mechanism of neurovascular coupling is not clear. It is also not known whether it is altered in neurologic diseases such as migraine. A method that would enable analysis of neurovascular coupling in humans is still to be discovered.

It has been shown that Trans cranial Doppler sonography (TCD) measures cerebral perfusion changes related to neuronal activation in a way comparable to firm.[1] 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.[2] A TCD study has observed an age-dependent decline of visually evoked cerebral blood flow velocity responses (VEFR).[4] Therefore, the attenuation of vascular responses during ageing could be attributed to decreased neurovascular coupling activity.

Migraine is considered a neurovascular disorder, in which both vascular and neuronal components play a pathophysiological role.[4,5] During visual stimulation, several functional neuroimaging methods including fMRI, positron emission tomography (PET) and TCD showed a localized increase in blood volume and blood flow velocity in the territory of posterior cerebral artery (PCA) in the patients with migraine during the headache-free period compared to healthy controls.[6–8] Empirically, however, without a corresponding index of neuronal electrical activity, any increase in blood flow observed with neuroimaging methods upon stimulation might have occurred through increase in neuronal activity, the neurovascular coupling mechanism, or both. Electrophysiological studies performed over the last years have demonstrated a dysfunction in cortical excitability in migraneurs during headache-free periods.[9] Studies of cortical excitability by transcranial magnetic stimulation have yielded conflicting results.[10] However, results obtained using habituation of pattern-reversal visual evoked potentials to explore cortical excitability changes induced by repetitive transcranial magnetic stimulation suggest decreased level of preactivation excitability, rather than hyperexcitability.[11]

The analysis would require a change of neuronal activity, and a noninvasive simultaneous monitoring of regional blood flow and neuronal activity. Neuronal activity could be noninvasively recorded by measuring visually evoked potentials (VEP), changed by applying different visual contrasts. At the same time, regional blood flow could be monitored by using TCD and analyzing VEFR. The aim of our study was to establish whether visual contrast affects VEFR, whether VEFR relate to VEP, and whether the relationship between VEP and VEFR is interictally altered in patients with migraine as well as between two different age groups.

Subjects and methods

The records were made from 40 healthy volunteers aged 48.7 ± 17.4 years (from 22 to 78 years) of both sexes (21 women and 19 men). The younger group (< 55 year) consisted of 20 subjects and older one (> 55 year) of 20 healthy individuals. Thirty patients with migraine aged 36.6 ± 10.4 years (from 19 to 51 years; 20 women and 10 men), were included in the study. The group of patients with migraine was further divided into subgroups of patients without aura (MwA) (16 pts.) and with aura (MA) (14 pts.). The diagnosis of migraine was made independently from the neurosonologist by a neurologist according to the International headache society (IHS) criteria (The international classification of headache disorders, 2004). Neither the clinical and neurological examination nor the ultrasound examination of cerebral and precerebral arteries showed any hemodynamically significant stenosis in any of the subjects. CT scans were done for the subjects older than 55 years of age, and did not show any significant changes. We also performed a mini-mental test, which was normal in all the subjects. The visual acuity was investigated with Snellen cards, with the subjects with refractive errors wearing their glasses during the recordings. All the subjects were asked not to drink

caffeine-containing beverages and to refrain from smoking on the test day. The volunteers on any sort of medication were excluded. In patients with migraine we applied a short questioner including: duration of migraine, number of attacks per month, number of days from the last attack, presence of aura, prophylactic treatment and characteristic of attacks.
Examinations were performed during headache-free intervals in a dark, quiet room at the same time of day, after an adaptation of at least 10 minutes. 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 and fixed their eyes on a small spot of red light in the centre of the computer screen.

Stimulus paradigm

The visual stimulation was applied using checkerboard on-set paradigm, which was presented by the computer screen. The distance between the subjects and the screen was 1 metre subtended 22 degrees of the visual angle. The stimulus 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/m^2. We changed the luminance (L) of the checks in order to get visual contrasts of 100 %, 10 % and 1 %. The 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.
The experimental session consisted of stimulus-eyes-opened and stimulus-eyes-closed periods. The stimulus-eyes-opened period lasted 70 seconds. During that period we presented the visual stimuli i.e. pattern on-set stimulus where the checkerboard pattern interchanged with diffuse white stimulus of equal mean luminance. The duration of the checkerboard pattern appearance was 200 ms and that of the diffuse white stimulus 500 ms. The stimulus frequency was 1.4 Hz. Therefore, exactly 100 VEP were obtained during the stimulus-eyes-opened period. The stimulus eyes-open period was repeated five times in a row at each visual contrast in order to get approximately 500 VEP for each visual contrast. The stimulus eyes-close period lasted 30 seconds. The order of the stimulus conditions was randomly varied for each subject.

VEFR recording

A multimodal recording of arterial blood pressure, heart rate, end-tidal carbon dioxide and arterial blood flow velocity was performed. The arterial pressure was continuously monitored with a blood pressure monitor (Colin 7000, Komaki-City, Japan). The end-tidal carbon dioxide was monitored with an infrared capnograph (Capnodig, Draeger: Lübeck, Germany). The heart rate was determined by processing the Doppler signal with the TCD8 commercial software (Multidop X4/TCD8; DWL Electronische System GmbH, Sipplingen, Germany). The arterial blood velocity was recorded in the left middle cerebral artery (MCA) and in the right posterior cerebral artery (PCA) through temporal acoustic windows with Multi-Dop X4 (DWL, Sipplingen, Germany), using 2 MHz transducers. The vessels were identified according to the criteria described elsewhere.[12] Confirming the vessels localisation, we considered anatomic landmarks, the direction of the flow and compression manoeuvres. The criterion for successful PCA insonation was a clear-cut flow velocity increase during the period when the subjects had their eyes opened as opposed to the period when their eyes were closed. The P2 segment of the PCA was always insonated since it proved to have a higher visual response than the P1 segment.[13] The MCA and the PCA were insonated at typical depths of 54 mm and 64 mm respectively. We tried to maintain a 10 mm depth difference between the MCA and the PCA.
The multimodal recording consisted of the basal period before the start of stimulation, and of the stimulation periods. We determined the mean amplitudes of the arterial blood flow velocities in the MCA (v_mMCA), in the PCA (v_mPCA), as well as the mean amplitudes of arterial pressure (MAP), heart rate (HR) and end tidal carbon dioxide (Et-CO_2), for the basal 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 with the TCD8 software, using a commercial algorithm according to the formula:

$$A_m = \int v d_t/(t_0 - t_1),$$

where A_m represents the mean amplitude of the variables (v) included in the measurements and t_0–t_1 is the time interval in which variable was integrated. The VEFR were defined as the difference between the v_mPCA at the basal conditions and the v_mPCA at the stimulus eyes-open periods at 1 %, 10 % and 100 % visual contrasts.

VEP recording

The cerebral evoked activity was recorded from the scalp by means of silver-silver chloride cup electrodes (10 mm diameter), fixed by contact paste. The resistance had been kept below 5kW. The electrodes were placed according to the recommendations of the International Society for Clinical Electrophysiology of Vision (ISCEV).[14] Three active electrodes were used, i.e. the Oz designated electrode placed 10 % of the inion-nasion distance above the inion, and the O1 and O2 designated electrodes 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 ms, and the sensitivity 10mV per division. The signal was subsequently led to a computer system, which also served as an on-line averager. Five hundred responses were commonly averaged and presented on the screen of an oscilloscope prior to being recorded on disc memory.

The analysis of the averaged responses of the on-set VEP, as detected from the active electrode at O1 position, was made off-line on a computer system by measuring the amplitudes in the interval of interest. We had designed a software which enabled us to calculate the mean absolute amplitude $|\hat{A}|$ of the VEP according to the formula:

$$|\hat{A}| = \Sigma |A| / n,$$

where A is the amplitude of the sample and n the number of samples during the chosen period. Amplitudes of the samples were measured from baseline. The sampling frequency was 1.67 sample/msec. The mean absolute amplitude was calculated for the interval from 50 to 200ms, which is a typical interval where early on-set VEP response occurs.[15]

Statistical methods

In order to evaluate relationships between VEFR and VEP, a model of linear regression was applied. All the statistical analyses were performed using the SPSS statistical software. The differences were considered significant when $p < 0.05$. In order to analyse the difference between two slopes of the regression curves, a t-test was applied.[16]

Results

In the study we tested the relationship between VEP and VEFR (Figure 1). Linear regression showed significant positive moderate association between VEP in VEFR ($r = 0.66$, $b = 0.55$, $p < 0.01$).

In order to evaluate the effect of ageing on neurovascular coupling we tested the relationships between the VEP and the VEFR in the younger as well as in the older subjects (Figure 2). The linear regression showed a significant positive association between the VEP and the VEFR ($r = 0.66$, $p < 0.01$) in the younger and older subjects ($r = 0.74$, $p < 0.01$). The regression coefficient (slope) in the younger subjects was significantly higher ($b = 0.54$; SE = 0.07) than that in the older subjects ($b = 0.40$; SE = 0.05) ($p < 0.01$).

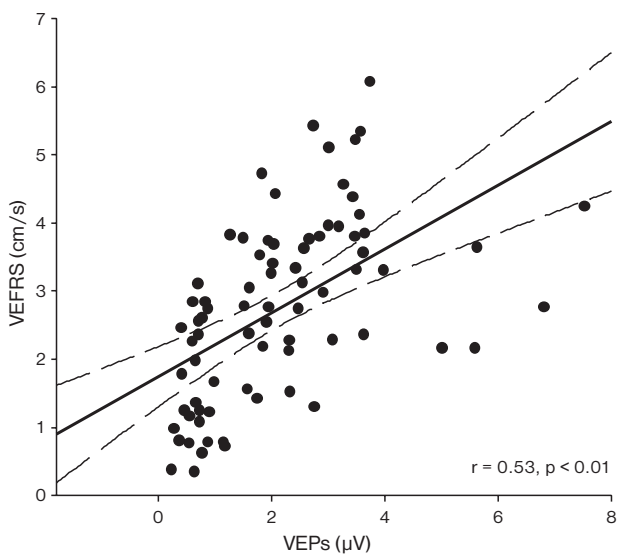

Figure 1. *The scatter plot between visually evoked cerebral blood flow velocity responses (VEFR) and visual evoked potentials (VEP) in healthy subjects.*

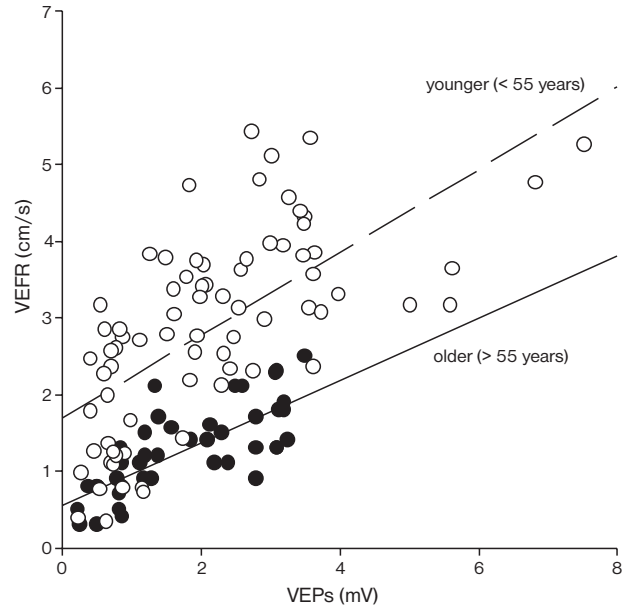

Figure 2. *The scatter plot of visual evoked potentials (VEP) amplitudes versus visually evoked cerebral blood flow velocity responses (VEFR) amplitudes. Linear regression line was fitted to the experimental data of younger (dash line) and older subjects (solid line). Linear regression coefficient for younger was b = 0.54 and significance p < 0.01. Linear regression coefficient for older was b = 0.40 and significance p <0.01. The difference between regression coefficients of older and younger subjects was significant (p < 0.01).*

In order to examine neurovascular coupling in migraine, we tested the relationship between the VEP and the VEFR in healthy controls and in patients with migraine (Figure 3). The linear regression analysis showed a positive correlation between the VEP in the VEFR ($r = 0.66$, $p < 0.01$) in both healthy controls and in patients with migraine ($r = 0.63$, $p < 0.01$). The regression coefficient (slope) in the group of patients with migraine was 0.88 (SE = 0.08) and in healthy controls 0.55 (SE = 0.07), which was statistically significantly different ($p = 0.04$). We did not find any significant differences between regression coefficients in MwA compared to MA ($p = 0.96$).

The other variables, i.e. the v_mMCA ($p = 0.11$), MAP ($p = 0.22$), Et-CO_2 ($p = 0.18$) and HR ($p = 0.17$), did not show significant differences along the measuring points in healthy subjects ($p = 0.11$, $p = 0.22$, $p = 0.18$, $p = 0.17$ respectively) as well as in patients with migraine ($p = 0.32$, $p = 0.42$, $p = 0.15$, $p = 0.26$ respectively). The differences between the both subgroups (v_mMCA: $p = 0.54$; MAP: $p = 0.42$; Et-CO_2: $p = 0.38$; HR: $p = 0.67$) were also not significant.

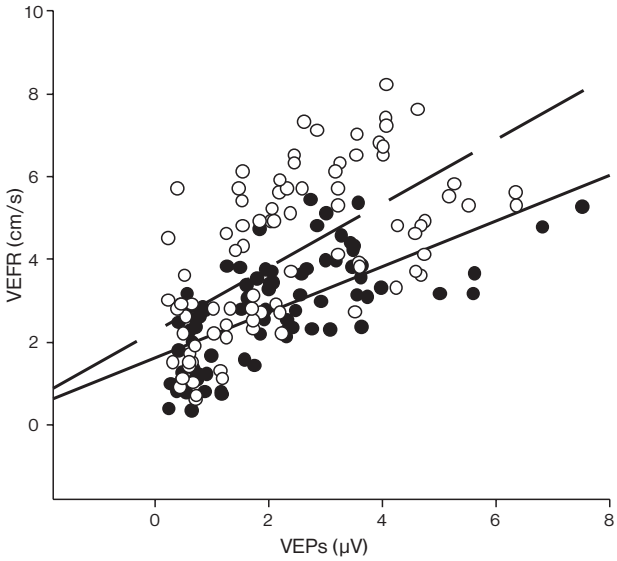

Figure 3. *The scatter plot between visually evoked cerebral blood flow velocity responses (VEFR) and visual evoked potentials (VEP) in patients with migraine (dotted line) and healthy subjects (solid line). The regression coefficients for migraine group is b = 0.88 (standard error = 0.08; p < 0.01) and healthy subjects b = 0.53 (standard error = 0.07; p < 0.01).*

Discussion

We tested the relationship between VEP and VEFR, which has showed significant linear association. The result supports findings of other studies explored the relationship between neuronal activation and vascular responses in humans and animals. Simultaneous measurements of neuronal activity and haemodynamic responses upon stimulation in animals showed that the vascular responses directly reflect an increase in neuronal activity, correlating in particular with local field potential, which represents the synchronized synaptic inputs of a given neuronal population.[17, 18] Accordingly, significant correlation between evoked potentials and CBF was found in animals.[19] They reported a linear relationship between N20P22 of the somatosensory evoked potentials (SEP) and BOLD-contrast fMRI in response to graded stimulus intensity in humans.[20] However, this study did not perform simultaneous measurement of SEP and fMRI. It was shown that the registration of electrophysiological response in the magnet is feasible, but the signal to noise ratio is decreased by simultaneous registration.[21] Combined approach using NIRS and simultaneously measured VEP amplitudes found linear relationship between the habituation in P100N135 amplitude and the habituation in the vascular parameters.[22] However, the method cannot ascertain that the volumes sampled are the same as for electrophysiological recordings and for the oxygenation changes measured by NIRS.

We found that the regression between VEP and VEFR is sufficiently approximated by linear equation. This is in agree with the similar studies in animals. In the rat the linear coupling between SEP amplitude and vascular response as measured by laser doppler flowmetry in response to different stimulation frequencies was found.[23] Similar study showed that activity-dependent increases in neuronal activity and cerebral blood flow were linearly coupled under defined conditions.[24] These findings support the usefulness of the presented approach with TCD to assess neurovascular coupling noninvasively in the human.

Simultaneous recording of VEFR and VEP in our study allowed us to test the relationship between VEP and VEFR in healthy subjects and patients with migraine. We found linear relation between the VEP and VEFR upon visual stimulation in both groups of subjects. This is in line with our recent observations that the association between VEP and VEFR upon visual stimulation with increasing contrast.[25] We found significantly higher regression coefficient (steeper slope) in patients with migraine compared to healthy subjects, which suggest a higher neurovascular coupling in patients with migraine interictally. There were no differences in neurovascular coupling between MA and MwA patients. The exact mechanism of increased neurovascular coupling in migraneurs is not known at present. Nitric oxide, a potent vasodilator of cerebrovascular smooth muscle, play an important role as mediator or modulator in the coupling of blood flow to cortical activation[26] as well as in the pathogenesis of migraine.[27] Therefore, it is tentative to speculate that the effect of nitric oxide is intrinsically increased in migraneurs. Increased neurovascular coupling in migraneurs could be associated also with altered serotoninergic activity, which affect simultaneously vascular activity as well as neuronal activity.[28] On the other hand, the genetic alterations of calcium channels could be resulted in increased activity of neurovascular coupling.[29]

We found a linear relationship between VEP and VEFR in younger as well as in older subjects. Our major finding was that the regression coefficient was significantly lower in older subjects as compared to younger ones. This finding suggested a diminished function of neurovascular coupling in older subjects. Although the studies have explored the relationship between evoked potentials and vascular responses detected by fMRI,[30] and near infrared spectroscopy (NIRS),[22] none of them has studied the effect of ageing on this relationship. An indirect study with TCD using control system analysis has suggested that neurovascular coupling mechanism is unaffected by moderate ageing as estimated by Doppler parameters.[31] In our study as well as in the other TCD studies that have also reported diminished responses in older subjects, however, the age of the subjects was importantly greater.[32]

We concluded that simultaneous recording of VEFR and VEP upon graded visual contrasts could allow an assessment of neurovascular coupling in both healthy and patients with migraine. It seems that higher vascular responses upon visual stimulation in patients with migraine than in healthy controls are due to an increase of neurovascular coupling activity in migraneurs. This could be an important phenomenon in migraine pathophysiology, which warrants

further studies. Simultaneous recording of VEFR and VEP to graded visual contrasts indicates diminished activity of neurovascular coupling in older subjects and has to be considered when interpreting the results of functional neuroimaging studies. The ageing process probably affects neurovascular coupling itself.

References

1. Borbely K, Gjedde A, Nyary I, Czirjak S, Donauer N, Buck A. Speech activation of language dominant hemisphere: a single-photon emission computed tomography study. Neuroimage 2003; 20: 987–94.
2. Langenecker SA, Nielson KA, Rao SM. fMRI of healthy older adults during Stroop interference. Neuroimage 2004; 21: 192–200.
3. Panczel G, Daffertshofer M, Ries S, Spiegel D, Hennerici M. Age and stimulus dependency of visually evoked cerebral blood flow responses. Stroke 1999; 30: 619–23.
4. Bolay H, Reuter U, Dunn AK, Huang Z, Boas DA, Moskowitz MA. Intrinsic brain activity triggers trigeminal meningeal afferents in a migraine model. Nat Med 2002; 8: 136–42.
5. Goadsby PJ, Lipton RB, Ferrari MD. Migraine—current understanding and treatment. N Engl J Med 2002; 346: 257–70.
6. Thie A, Fuhlendorf A, Spitzer K, Kunze K. Transcranial Doppler evaluation of common and classic migraine. Part I. Ultrasonic features during the headache-free period. Headache 1990; 30: 201–8.
7. Backer M, Sander D, Hammes MG, Funke D, Deppe M, Conrad B, Tolle TR. Altered cerebrovascular response pattern in interictal migraine during visual stimulation. Cephalalgia 2001; 21: 611–6.
8. Vincent M, Pedra E, Mourao-Miranda J, Bramati IE, Henrique AR, Moll J. Enhanced interictal responsiveness of the migraineous visual cortex to incongruent bar stimulation: a functional MRI visual activation study. Cephalalgia 2003; 23: 860–8.
9. Schoenen J, Ambrosini A, Sandor PS, Maertens de Noordhout A. Evoked potentials and transcranial magnetic stimulation in migraine: published data and viewpoint on their pathophysiologic significance. Clin Neurophysiol 2003; 114: 955–72.
10. Brighina F, Piazza A, Daniele O, Fierro B. Modulation of visual cortical excitability in migraine with aura: effects of 1 Hz repetitive transcranial magnetic stimulation. Exp Brain Res 2002; 145: 177–81.
11. Bohotin V, Fumal A, Vandenheede M, Gerard P, Bohotin C, Maertens de Noordhout A, Schoenen J. Effects of repetitive transcranial magnetic stimulation on visual evoked potentials in migraine. Brain 2002; 125: 912–22.
12. Aaslid R. Visually evoked dynamic blood flow responses of the human cerebral circulation. Stroke 1987; 18: 771–5.
13. Panczel G, Daffertshofer M, Ries S, Spiegel D, Hennerici M. Age and stimulus dependency of visually evoked cerebral blood flow responses. Stroke 1999; 30: 619–23.
14. Harding GF, Odom JV, Spileers W, Spekreijse H. Standard for visual evoked potentials 1995. The International Society for Clinical Electrophysiology of Vision. Vision Res 1996; 36: 3567–72.
15. Di Russo F, Martinez A, Sereno MI, Pitzalis S, Hillyard SA. Cortical sources of the early components of the visual evoked potential. Hum Brain Mapp 2002; 15: 95–111.
16. Glantz SA. Primer of applied regression and analysis of variance. New York: McGraw-Hill Inc; 1998. p. 28.
17. Mathiesen C, Caesar K, Akgoren N, Lauritzen M. Modification of activity-dependent increases of cerebral blood flow by excitatory synaptic activity and spikes in rat cerebellar cortex. J Physiol 1998 15; 512: 555–66.
18. Logothetis NK, Pauls J, Augath M, Trinath T, Oeltermann A. Neurophysiological investigation of the basis of the fMRI signal. Nature 2001; 412: 150–7.
19. Ogawa S, Lee TM, Stepnoski R, Chen W, Zhu XH, Ugurbil K. An approach to probe some neural systems interaction by functional MRI at neural time scale down to milliseconds. Proc Natl Acad Sci USA 2000; 97: 11026–31.
20. Arthurs OJ, Williams EJ, Carpenter TA, Pickard JD, Boniface SJ. Linear coupling between functional magnetic resonance imaging and evoked potential amplitude in human somatosensory cortex. Neuroscience 2000; 101: 803–6.
21. Bonmassar G, Schwartz DP, Liu AK, Kwong KK, Dale AM, Belliveau JW. Spatiotemporal brain imaging of visual-evoked activity using interleaved EEG and fMRI recordings. Neuroimage 2001; 13: 1035–43.
22. Obrig H, Israel H, Kohl-Bareis M, Uludag K, Wenzel R, Muller B, Arnold G, Villringer A. Habituation of the visually evoked potential and its vascular response: implications for neurovascular coupling in the healthy adult. Neuroimage 2002; 17: 1–18.
23. Ngai AC, Jolley MA, D'Ambrosio R, Meno JR, Winn HR. Frequency-dependent changes in cerebral blood flow and evoked potentials during somatosensory stimulation in the rat. Brain Res 1999; 837: 221–8.
24. Norup Nielsen A, Lauritzen M. Coupling and uncoupling of activity-dependent increases of neuronal activity and blood flow in rat somatosensory cortex. J Physiol 2001; 533: 773–85.
25. Zaletel M, Strucl M, Rodi Z, Zvan B. The association between visually evoked potentials and visually evoked flow reponses. Cerebrovasc Dis 2003; 16 suppl 2: 3.
26. Lindauer U, Megow D, Matsuda H, Dirnagl U. Nitric oxide: a modulator, but not a mediator, of neurovascular coupling in rat somatosensory cortex. Am J Physiol 1999; 277: H799–811.
27. Sances G, Tassorelli C, Pucci E, Ghiotto N, Sandrini G, Nappi G. Reliability of the nitroglycerin provocative test in the diagnosis of neurovascular headaches. Cephalalgia 2004; 24: 110–9.
28. Goadsby PJ, Knight Y. Inhibition of trigeminal neurons by intravenous administration of the serotonin (5HT)1B/D receptor agonist zolmitriptan (311C90): are brain stem sites therapeutic target in migraine? Pain 1996; 67: 355–9.
29. Estevez M, Gardner KL. Update on the genetics of migraine. Hum Genet. 2004; 114: 225–35.
30. D'Esposito M, Zarahn E, Aguirre GK, Rypma B. The effect of normal aging on the coupling of neural activity to the bold hemodynamic response. Neuroimage 1999; 10: 6–14.
31. Rosengarten B, Aldinger C, Spiller A, Kaps M. Neurovascular coupling remains unaffected during normal aging. J Neuroimaging 2003; 13: 43–7.
32. Niehaus L, Lehmann R, Roricht S, Meyer BU. Age-related reduction in visually evoked cerebral blood flow responses. Neurobiol Aging 2001; 22: 35–8.

Accepted 2008-02-16