Multiresolution analysis of pathological changes in cerebral venous dynamics in newborn mice with intracranial hemorrhage: adrenorelated vasorelaxation

2014 Physiol. Meas. 35 1983

(http://iopscience.iop.org/0967-3334/35/10/1983)

IOP Publishing | Institute of Physics and Engineering in Medicine Physiological Measurement

Physiol. Meas. **35** (2014) 1983–1999 doi:10.1088/0967-3334/35/10/1983

Multiresolution analysis of pathological changes in cerebral venous dynamics in newborn mice with intracranial hemorrhage: adrenorelated vasorelaxation

A N Pavlov[1,2,3], O V Semyachkina-Glushkovskaya[3,4], Y Zhang[3], O A Bibikova[3,4], O N Pavlova[1], Q Huang[3], D Zhu[3], P Li[3], V V Tuchin[1,3,5] and Q Luo[3]

[1] Department of Physics, Saratov State University, Astrakhanskaya Str. 83, Saratov, 410012, Russia
[2] Saratov State Technical University, Politehnicheskaya Str. 77, Saratov, 410054, Russia
[3] Wuhan National Laboratory for Optoelectronics, Britton Chance Center for Biomedical Photonics, Huazhong University of Science and Technology, Wuhan 430074, People's Republic of China
[4] Department of Biology, Saratov State University, Astrakhanskaya Str. 83, Saratov, 410012, Russia
[5] University of Oulu, Optoelectronics and Measurement Techniques Laboratory, PO Box 4500, Oulu, FIN-90014, Finland

E-mail: pavlov.lesha@gmail.com

Received 7 February 2014, revised 4 May 2014
Accepted for publication 2 June 2014
Published 19 September 2014

Abstract

Intracranial hemorrhage (ICH) is the major problem of modern neonatal intensive care. Abnormalities of cerebral venous blood flow (CVBF) can play a crucial role in the development of ICH in infants. The mechanisms underlying these pathological processes remain unclear; however it has been established that the activation of the adrenorelated vasorelaxation can be an important reason. Aiming to reach a better understanding of how the adrenodependent relaxation of cerebral veins contributes to the development of ICH in newborns, we study here the effects of pharmacological stimulation of adrenorelated dilation of the sagittal sinus by isoproterenol on the cerebral venous hemodynamics. Our study is performed in newborn mice at different stages of ICH using the laser speckle contrast imaging and wavelet analysis of the vascular dynamics of CVBF. We show that the dilation of the sagittal sinus with the decreased velocity of blood flow presides to the stress-induced ICH in newborn mice. These morphofunctional vascular changes are accompanied by an increased variance of the wavelet-coefficients in the

areas of endothelial and non-endothelial (K_{ATP}-channels activity of vascular muscle) sympathetic components of the CVBF variability. Changes in the cerebral venous hemodynamics at the latent stage of ICH are associated with a high responsiveness of the sagittal sinus to isoproterenol quantifying by wavelet-coefficients related to a very slow region of the frequency domain. The obtained results certify that a high activation of the adrenergic-related vasodilatory responses to severe stress in newborn mice can be one of the important mechanisms underlying the development of ICH. Thus, the venous insufficiency with the decreased blood outflow from the brain associated with changes in the endothelial and the sympathetic components of CVBF-variability can be treated as prognostic criteria for the risk of ICH during the first days after birth.

Keywords: cerebrovascular dynamics, laser speckle contrast imaging, wavelet-analysis

(Some figures may appear in colour only in the online journal.)

1. Introduction

Intracranial hemorrhage (ICH) accounts for 39–54% of all pediatric strokes representing a major problem of modern neonatal intensive care (Lo 2011). The pathogenesis of ICH is multifactorial but nevertheless it was established that the disturbance of the cerebral blood flow (CBF) contributes to ICH (Perlman *et al* 1983). The role of pathological changes in CBF still remains disputable (Ballabh 2010). It is unclear whether the abnormalities in CBF are the prognostic criteria for ICH or the critical alterations in CBF occur due to the presence of the brain hemorrhage itself (Ment *et al* 1984, Wong *et al* 2008). Recent studies (Meek *et al* 1999, Munro *et al* 2004) supposed that low CBF is the key factor for severe ICH. Therefore, the monitoring of CBF is of high importance in neonatal and pediatric medicine (Ment *et al* 1984, Ballabh 2010, Azhan and Wong 2012). Low CBF in neonates with ICH is often associated with hypotension being one of the main reasons for the adverse neurodevelopmental outcome, an increase in the brain injury and mortality of neonates after ICH (Munro *et al* 2004, Di Gennaro *et al* 2011, Azhan and Wong 2012). Mechanisms responsible for the critical decreases in CBF in infants with ICH and the neonatal hypotension are still not established. It is assumed that abnormalities in the cerebral venous blood flow (CVBF) can play a crucial role in these pathological processes. Indeed, ICH in newborns is primary venous (Ghazi-Birry *et al* 1997). The majority of cases of the spontaneous intracranial hypotension are related to the suppression of the blood outflow from the brain and the elevations of cerebral venous blood pressure that contribute to the occurrence of ICH in newborns (Volpe 2008, Gupta *et al* 2009). Our recent studies (Semyachkina-Glushkovskaya *et al* 2013a, Pavlov *et al* 2014) performed in newborn rats with stress-induced ICH showed that the dilation of the superior sagittal sinus with the fall of velocity of blood flow precedes the incidence of ICH. In adult rats with ICH we found similar pathological changes in cerebral hemodynamics observed at the transient state of ICH while the alteration in the arterial tree was detected only in rats with ICH (Semyachkina-Glushkovskaya *et al* 2013b).

Cerebral vessels have a rich sympathetic innervation distributed widely throughout the cerebral circulation (Edvinsson *et al* 1993, Edvinsson and Hamel 2002). The sympathetic

nervous system plays a key role in the regulation of the myogenic tone of the cerebral arteries and veins from the early stages of ontogeny (Bevan *et al* 1981, McCalden 1981, Busija and Heistad 1984). The adrenorelated vasorelaxation due to the activation of non-innervated β_2-adrenoreceptors (Lands *et al* 1967, Wilffert *et al* 1982) is presumably the important mechanism for the dilation of different types of vessels including the cerebral arteries and veins (Garland *et al* 2011, Hare *et al* 2006). Pathological alterations in the molecular mechanism, by which β_2-adrenoreceptors provide Gs-protein-dependent activation of the protein kinase A and β-arrestin-mediated signaling pathways, trigger stress-induced circulatory disorders (Hara *et al* 2011). It is crucial, however, that little information is known about the role of adrenorelated relaxation of cerebral vessels in the development of ICH.

Aiming to characterize how the adrenodependent relaxation of cerebral veins contributes to the development of ICH in newborns we apply here the multiresolution analysis based on the discrete wavelet-transform (DWT) (Meyer 1993a, 1993b, Daubechies 1992, Addison 2002). This mathematical tool is widely used in many scientific and technical applications if one requires quantifying multiscale dynamics of time-varying systems. The multiresolution analysis of temporal dynamics with DWT is able to reveal time scales associated with the most distinct changes in CVBF at transformations of normal physiological processes into pathological dynamics (Thurner *et al* 1998, Dremin *et al* 2001). As a consequence, it becomes possible to associate such changes with different physiological control mechanisms (in our case, with endothelial and non-endothelial sympathetic components of the CVBF variability). This analysis provides information about the variability of wavelet-coefficients reflecting the adaptation abilities of living organisms or the strength of physiological regulation (Thurner *et al* 1998, Dremin *et al* 2001, Pavlov *et al* 2013). By analogy with the classical spectral analysis, this approach allows the characterizing system's responses in various areas of the frequency domain. However, it has advantages when dealing with short and nonstationary data. Thus, our recent study of stress-induced phenomena with wavelet-based techniques allowed us to reveal distinct types of stress-induced reaction that were not recognized by spectral or correlation analysis (Pavlov and Anishchenko 2007).

As an experimental technique for studying CVBF we use laser speckle contrast imaging (LSCI) (Briers and Webster 1996, Briers 2001, Liu *et al* 2008, Boas and Dunn 2010, Tuchin 2013). It provides an opportunity to analyze blood flow in vessels with high spatio-temporal resolution. Laser speckle represents a pattern that is formed due to the scattering of the coherent light from the analyzed object. For rough surfaces, differences of the optical path for the reflected waves influences the light intensity at the observation point. When these differences become larger compared with the light wavelength, the intensity demonstrates significant variations: it increases for in-phase waves and decreases for anti-phase waves. The resulting intensity in an observation point changes in time because the scattering particles of the blood move through the vessel. Analysis of temporal changes of the speckle pattern provides a way to quantify the dynamics of CVBF. For this purpose, changes of the contrast are recalculated into the velocity of the blood flow. Recently, the LSCI-method has found many successful applications in medicine including the analysis of perfusion in retina (Briers and Fercher 1982, Suzuki *et al* 1991), skin (Forrester *et al* 2002, Stewart *et al* 2006) and other tissues (Bray *et al* 2006, Forrester *et al* 2004).

In order to reach a better understanding of how the adrenodependent relaxation of cerebral veins contributes to the development of ICH in newborns, we analyze the effects of the pharmacological stimulation of adrenorelated dilation of the sagittal sinus by isoproterenol on cerebral venous hemodynamics.

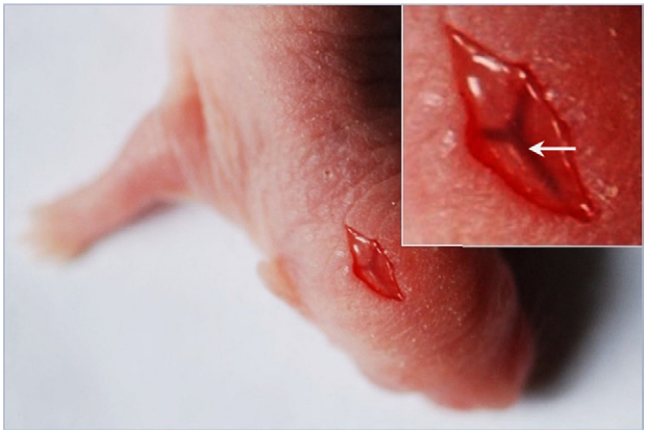

Figure 1. Sagittal sinus (arrowed) in newborn mouse observed through the fontanel.

2. Materials and methods

2.1. Subject

Experiments were performed in full term newborn mice ($n=29$) 2–3 d old. All procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The experimental protocols were approved by the Committee for the Care and Use of Laboratory Animals at the Huazhong University of Science and Technology. The mice were housed at 25 ± 2 °C, 55% humidity, and with a 12 : 12 h light/dark cycle.

2.2. Measurements of CVBF

The anesthetized mice (isoflurane—inhalant anesthetic) with fixed head and scalp incision (the dura was left intact) were immobilized. Anesthetic depth was assessed by periodically monitoring the rear foot reflex. The measurement of CVBF with LSCI was performed through the fontanel with a focus on the superior sagittal sinus figure 1. Relative changes in CVBF were calculated as a percentage of the baseline. From each experiment we extracted 2–3 time series characterizing the velocity in different parts of the sagittal sinus in arbitrary units.

2.3. Experimental procedure

Protocol 1: study of the harmful effect of infrasound on cerebral venous blood flow in newborn mice. To induce ICH, the newborn mice underwent an intermittent infrasound (10 Hz, 120 dB): 10 s—the sound, then 60 s—the interruption, and this cycle was repeated during 2 h. This procedure was performed in the Plexiglas chamber (the volume—2000 sm^3) absorbing and amplifying the deleterious effects of infrasound on the mice. Recording of CVBF was carried out: (1) in mice under normal conditions ($n=10$); (2) in mice ($n=10$) at 4 h after the influence of infrasound (the transient stage of ICH); (3) in mice ($n=9$) at 24 h after the stress-off. These stages have been detected in our previous studies on newborn and adult rats using Doppler optical coherence tomography and histological assay (Semyachkina-Glushkovskaya *et al* 2013a, 2013b).

Protocol 2: study of the pharmacological stimulation of β_2-adrenodependent vasorelaxation on CVBF in newborn mice under normal conditions and at different stages of ICH. After a baseline measurement during 10 min, changes in CVBF to the stimulation of β_2-adrenodependent vasorelaxation were evaluated by intravenous (through the tail vein) administration of isoproterenol (Sigma, 0.05 g kg^{-1}). CVBF was recorded continuously during the 30 min after the isoproterenol injection. In this protocol we used the same groups indicated in the protocol 1. The control group included newborn mice treated with physiological saline under normal conditions ($n=8$), at the transient stage of ICH ($n=8$) and during the development of ICH ($n=8$).

2.4. Statistical analysis

Results are presented as the mean ± standard error of the mean (SEM). The differences from the initial level in the same group were evaluated by the Wilcoxon test. Inter-group differences were evaluated using the Mann-Whitney test and ANOVA-2 (post hoc analysis with Duncan's rank test). Significance levels were set at $p<0.05$ for all analyses.

2.5. Multiresolution analysis

Multiresolution analysis (Meyer 1993a, 1993b) is a mathematical approach that assumes the approximation of a signal $f(t)$ by a series of quite simple functions $\varphi_{m,k}(t)$ and $\psi_{j,k}(t)$:

$$f(t)=\sum_k s_{m,k}\ \varphi_{m,k}(t)+\sum_{j\geqslant m}\sum_k d_{j,k}\psi_{j,k}(t)\,. \tag{1}$$

Equation (1) is valid for any $f(t)\in L^2(R)$ on any resolution level m. The functions $\varphi_{m,k}(t)$ and $\psi_{j,k}(t)$ are treated as a set of low-frequency and high-frequency filters, respectively, that are constituted by the translation and the dilation of the scaling function $\varphi(t)$ and the wavelet $\psi(t)$:

$$\varphi_{m,k}=2^{m/2}\varphi(2^m t-k)\,,\quad \psi_{j,k}=2^{j/2}\psi(2^j t-k)\,. \tag{2}$$

The used functions should satisfy the conditions of localization and regularity. Besides, an additional requirement of a zero mean value is introduced for wavelets. The functions $\varphi(t)$ and $\psi(t)$ are selected in such a way that they can fully be represented by their values at rescaling with the factor 2^j, i.e., at the transition to another level of resolution. For the simplest, the Haar wavelet, the approximation of $f(t)$ by $\varphi(t)$ is interpreted as the histogram approach (a representation of the signal by averaged mean values within some time intervals) while the wavelets $\psi(t)$ add details to this approximation on smaller levels of resolution (Meyer 1993a, Addison 2002). Transition from the level j to $j+1$ is equivalent to a changing of t by $2t$. The relations between the considered functions at different levels of resolution are described by the expressions:

$$\varphi(t)=\sqrt{2}\sum_{k=0}^{M-1}h_k\ \varphi(2t-k)\,,\quad \psi(t)=\sqrt{2}\sum_{k=0}^{M-1}h_{M-1-k}\ \varphi(2t-k)\,. \tag{3}$$

The filter coefficients h_k are estimated numerically from general properties of scaling functions and wavelets such as, e.g., the orthogonality of the translated scaling functions $\varphi(t)$ and $\varphi(t-k)$, the orthogonality of wavelets $\psi(t)$ and scaling functions $\varphi(t-k)$, and the regularity properties of wavelets, etc.

Taking into account these circumstances and introducing different restrictions to wavelets (for instance, how regular should the function $\psi(t)$ be), various sets of filters h_k, $k=1$, …,

$M-1$ are estimated. For small M, the coefficients h_k are given by analytic expressions. As an example, the wavelet D^4 related to the well-known Daubechies wavelets (Daubechies 1992) is represented by the coefficients:

$$h_0 = \frac{1}{4\sqrt{2}}(1+\sqrt{3}), \quad h_1 = \frac{1}{4\sqrt{2}}(3+\sqrt{3}),$$
$$h_2 = \frac{1}{4\sqrt{2}}(3-\sqrt{3}), \quad h_3 = \frac{1}{4\sqrt{2}}(1-\sqrt{3}). \tag{4}$$

For more regular wavelets, the values h_k are estimated from solutions of algebraic equations of larger power and can be found numerically with the required accuracy. Thus, the Daubechies wavelet D^8 is given by the coefficients h_k:

$$\begin{aligned} h_0 &= -0.0757657, \quad h_1 = -0.0296355, \\ h_2 &= 0.49761866, \quad h_3 = 0.80373875, \\ h_4 &= 0.29785779, \quad h_5 = -0.0992195, \\ h_6 &= -0.0126039, \quad h_7 = 0.03222310. \end{aligned} \tag{5}$$

Within the multiresolution analysis, the signal $f(t)$ is decomposed using a selected basis of wavelets. The coefficients $d_{j,k}$ of this decomposition performed according to equation (1) carry important information about the structure of $f(t)$ on different independent scales. This analysis is sometimes called the 'mathematical microscope' because it is able to reveal features of signals structure for a wide range of resolution levels: from large to small scales. Within this interpretation, the selection of the basis function $\psi(t)$ is similar to the choice of an objective for a microscope that allows (or does not allow) the revealing of necessary details.

Because the signal $f(t)$ demonstrates temporal variations that are more pronounced for non-stationary regimes, the estimated coefficients $d_{j,k}$ also fluctuate in time. A good statistical measure of variability of the wavelet coefficients is the standard deviation of $d_{j,k}$ estimated as a function of the scale

$$\sigma(j,N) = \sqrt{\frac{1}{N}\sum_{k=0}^{N-1}[d_{j,i} - \langle d_{j,i} \rangle]^2}. \tag{6}$$

where N is the number of wavelet-coefficients at the level j. This measure was successfully used, e.g., in cardiology providing clinically significant diagnostics of heart failures based on beat-to-beat intervals (Thurner *et al* 1998). An important conclusion of the work (Thurner *et al* 1998) consisted of the possibility of revealing time scales associated with pathological changes and, therefore, in establishing control mechanisms responsible for the development of this pathology. In order to apply the diagnostics abilities of the multiresolution analysis, it should be appropriately 'tuned', i.e., the optimal wavelet function $\psi(t)$ needs to be selected. This selection is the required step for an analysis of LSCI-data because an unsuccessful choice of $\psi(t)$ will not allow for the revealing of necessary details.

3. Results

3.1. Harmful effects of infrasound on CVBF in newborn mice

At the first step, we analyzed the changes in the cerebral venous hemodynamics in newborn mice at different stages of the stress-induced ICH: at 4 h after sound stress (the transient

Table 1. The diameter (μm) of the sagittal sinus in newborn mice before and after the physiological saline and isoproterenol injection under normal conditions and at different stages of ICH.

	Healthy mice	Stressed mice (4 h after infrasound influences, transient stage)	Stressed mice (24 h after infrasound influences, incidence of ICH)
Basal levels before physiological saline administration	276 ± 15	346 ± 15[b]	372 ± 21[b]
Basal levels after physiological saline administration	279 ± 11	341 ± 13[b]	370 ± 15[b]
Isoproterenol administration	334 ± 12[a]	469 ± 22[a,b]	380 ± 17[b]

$p<0.05$ versus: [a] basal levels; [b] healthy mice.

stage of ICH without ICH) and at 24 h after stress-off (the incidence of ICH). Our results demonstrated that the development of ICH in newborn mice is characterized by essential changes in the parameters of cerebral venous circulation. Indeed, the transient stage of ICH is accompanied by an increase in the diameter of the sagittal sinus by about 25% ($p<0.05$) and a decrease in the velocity of blood flow by about 15% ($p<0.05$) compared with the normal state. The incidence of ICH is associated with more pronounced changes in the diameter of the sagittal sinus. Thus, this parameter increased by 34% ($p<0.05$) in newborn mice with ICH vs. healthy animals. In this period the velocity of blood flow decreased by 17% ($p<0.05$) that is almost the same as in mice at the transient stage. Table 1 shows the basal values of the diameter of the sagittal sinus. Figure 2(*a*) illustrates relative changes in the velocity of blood flow at rest and at different stages of the development of ICH. Temporal variations of CVBF associated with observation points inside the sagittal sinus are shown in figure 3.

3.2. Effect of the pharmacological stimulation of β_2-adrenodependent vasorelaxation on CVBF in newborn mice under normal conditions and at different stages of ICH

At the second stage, we studied the effect of agonist of β_2-adrenoreceptors—isoproterenol on the cerebral venous circulation at rest and at different stages of ICH. In healthy newborn mice, isoproterenol administration caused an increase in the diameter of the sagittal sinus by 19% ($p<0.05$) and a decrease in the velocity of blood flow by 23% ($p<0.05$). The responsiveness of the sagittal sinus to isoproterenol significantly increased at the transient stage of ICH figure 2(*b*). Actually, the diameter of the sagittal sinus increased by 37% ($p<0.05$), and the velocity of blood flow decreased by 38% ($p<0.05$). Let us mention that mice with ICH did not demonstrate essential changes in the diameter of the sagittal sinus after the isoproterenol injection table 1. However, the velocity of blood flow in this venous vessel decreased by about 30% ($p<0.05$) in mice with ICH figure 2(*b*). Administration of the physiological saline did not show any alterations in the diameter of the sagittal sinus and the velocity of blood flow in all mice (table 1).

3.3. Multiresolution analysis of changes in CVBF during the stimulation of β_2-adrenodependent vasorelaxation by isoproterenol at different stages of ICH

Before the application of the multiresolution analysis to experimental data recorded in newborn mice, a preliminary data preprocessing was performed. By analogy with the fast Fourier transform, it is convenient to operate with the number of data points equal to 2^n because

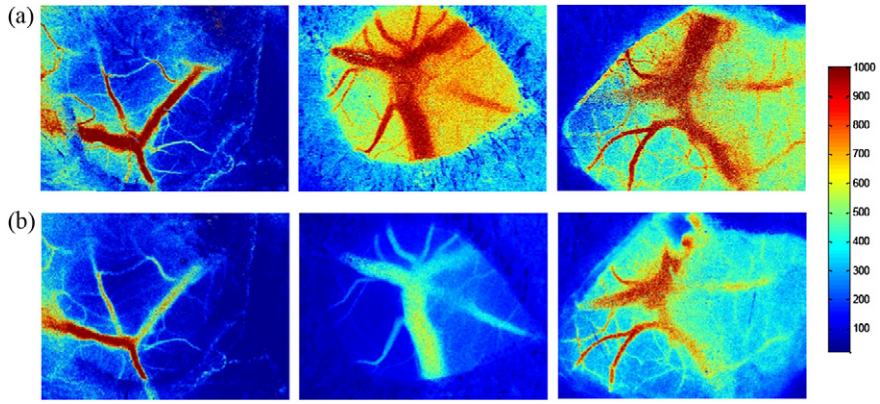

Figure 2. Effects of infrasound (*a*) and isoproterenol administration (*b*) on CVBF in the area of sagittal sinus in newborn mice. The distribution of CVBF was measured by the LSCI before stress (left), and 4 h (middle) and 24 h (right) after the stress.

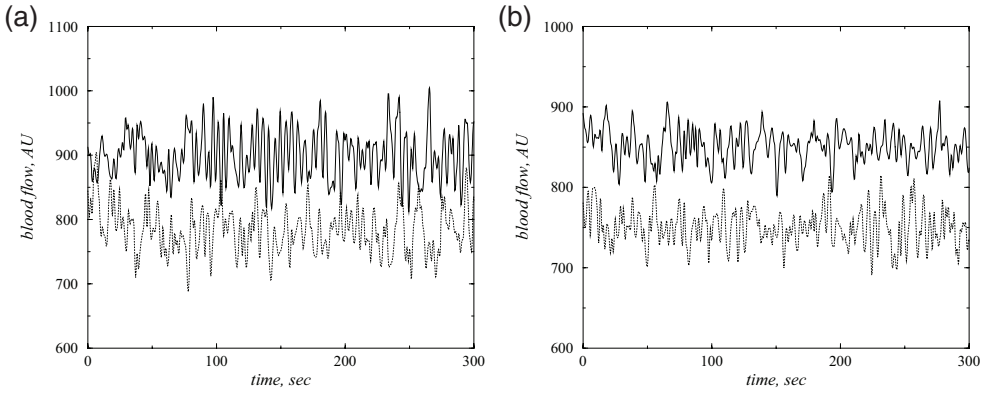

Figure 3. Temporal variations of CVBF associated with observation points inside the sagittal sinus: (*a*) for figure 2(*a*) before stress (solid line) and 24 h after stress (dotted line); (*b*) for figure 2(*b*) before stress (solid line) and 24 h after stress (dotted line).

transitions to each next level of resolution correspond to the rescaling of the time axis with the factor 2. For this purpose original data ($N=300$) were interpolated by cubic splines to get 2^{10} data points. The interpolation changed time intervals between samples from 1 s to about 0.3 section Let us note that this interpolation formally increases the range of frequencies for spectral analysis however we cannot be sure in correct spectral estimations in the extended frequency area. Due to this, our further analysis is restricted by the range below 0.5 Hz.

Within the multiresolution analysis, each next level of resolution $j+1$ corresponds to a 2 times smaller (compared to j) interval of time covered by the wavelet-function, i.e., to 2 times higher frequencies of rhythmic contributions. The considered scales can be associated with physiological control mechanisms because it was established, e.g., that the region of 0.25–0.75 Hz is associated with sympathetic regulation, the region 0.75–3.0 Hz corresponds to influences of parasympathetic nervous system and breathing, slower contributions (<0.25 Hz) are caused by endothelial factors (NO), intrinsic sympathetic mechanisms (without nervous regulation), etc (Stauss 2013a, 2013b). In our case, very slow

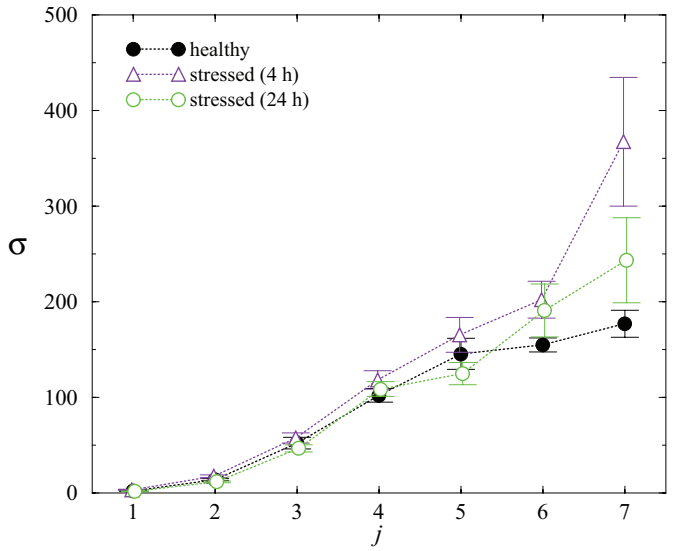

Figure 4. Analysis of the harmful effect of infrasound on CVBF in newborn mice with the variance of wavelet-coefficients associated with different levels of resolution. Analysis was performed with the Daubechies wavelet D^8. Deviation bars characterize the variability of the value σ for different mice.

mechanisms are associated with fluctuations in the experimental data at the levels $j \leqslant 6$, while the sympathetic nervous system corresponds to the range $6 \leqslant j \leqslant 7$. Thus, for $j = 6$ both mechanisms can influence the statistics of wavelet-coefficients. We do not consider levels $j > 7$ as corresponding to the frequencies $f > 0.5$ Hz due to the considered resolution of the LSCI-technique.

At the first step we analyzed the harmful effect of infrasound on CVBF in newborn mice with the multiresolution analysis. We applied the symmetric Daubechies wavelet D^8, it being the most widely used in practical studies of the Daubechies function. Figure 4 illustrates the variances of the wavelet-coefficients associated with different scales j for healthy mice and stressed animals at the different stages of ICH development: 4 h after the infrasound influences (the transient stage without ICH) and 24 h after sound stress (the incidence of ICH). Following figure 4, the transient stage of ICH is accompanied by the increased variance of wavelet-coefficients at the levels of resolution $j = [3;7]$ ($p < 0.05$). This effect becomes less pronounced after the incidence of ICH (24 h after stress).

Aiming to analyze responses to the administration of isoproterenol, we further estimated differences between the standard deviations of the wavelet-coefficients $d_{j,k}$ corresponding to the states before (1) and after the drug (2), i.e. the values $\sigma_2 - \sigma_1$. The experimental data of relative blood flow are quite nonstationary and their statistical characteristics (mean value, standard deviation, etc.) fluctuate in time. Analysis of control mechanisms responsible for rhythmic contributions at different time scales supposes a band-pass filtering of the data with the further study of filtered signals. Unlike the classical spectral analysis such filtering is automatically realized within the multiresolution technique.

Figure 5 illustrates a comparison of reactions to isoproterenol between the healthy mice and stressed animals (4 h after the stress). Healthy mice demonstrate a more pronounced reaction consisting of a reduced variability of the wavelet-coefficients (stronger regulation) at the

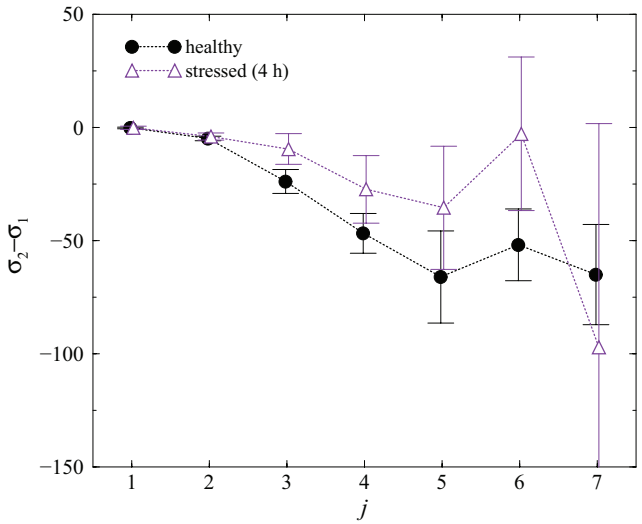

Figure 5. Reactions to isoproterenol for healthy mice and for stressed animals (4 h after the stress) characterized with the multiresolution analysis. Results are given for the Daubechies wavelet D^8.

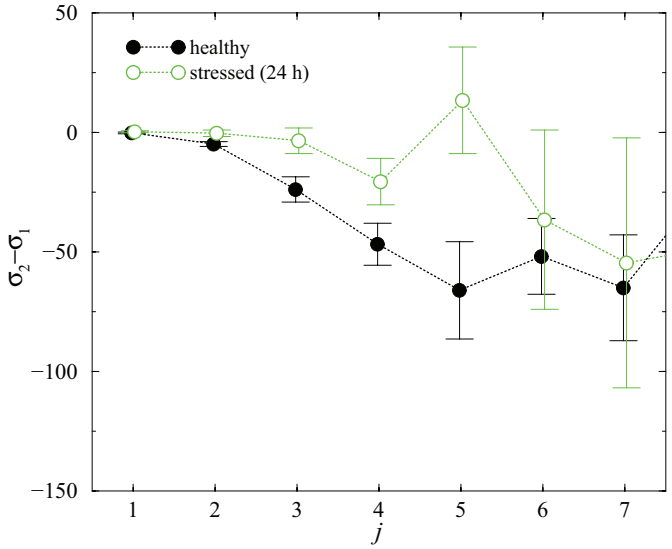

Figure 6. Reactions to isoproterenol for healthy mice and for stressed animals (24 h after the stress) characterized with the multiresolution analysis. Results are given for the Daubechies wavelet D^8.

levels of resolution $j=[3;6]$ ($p<0.05$) associated with very slow control mechanisms. This reaction is the most essential at the level $j=5$. At the level $j=7$ we did not reveal pronounced changes. Thus we can conclude that the reaction to isoproterenol is fairly weak for sympathetic regulation.

Reactions to isoproterenol between healthy mice and animals with stress-induced ICH (24 h after the stress) are significantly more pronounced in figure 6. By analogy with figure 4, the

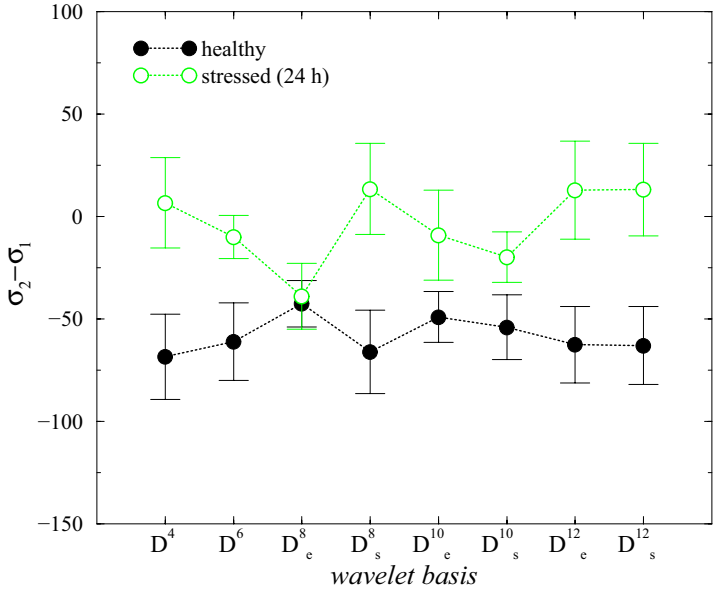

Figure 7. A comparison of reactions to isoproterenol for healthy mice and for animals with ICH (24 h after stress) with different wavelet bases including symmetric ('s') and asymmetric ('e') wavelets. The upper index characterizes the length of the wavelet. Results are given for the resolution level $j=5$.

main distinctions are associated with the scale $j=5$. The distinctions are significant ($p<0.05$) in the area of very slow regulatory mechanisms ($j=[3;5]$). They are less expressed for $j=6$, where the interplay between very slow and slow regulatory mechanisms can occur. Again, we do not observe inter-group changes for $j=7$. Thus, healthy animals show a reduced variability of wavelet-coefficients corresponding to a stronger regulation by very slow control mechanisms. However, mice with stress-induced ICH demonstrate almost unchanged variability of wavelet-coefficients associated with the corresponding physiological control. These observations are important for a better understanding of stress-induced phenomena in the brain circulation. Measures shown in figure 6, allow us to conclude about the essential potential of the multiresolution analysis as an approach that provides a characterization of individual physiological control mechanisms besides simple diagnostics of changes in the structure of the experimental time series.

In the performed study we used the symmetric Daubechies wavelet D^8 representing the most widely used wavelet function. Among wavelets with the relatively small support M, the wavelet D^8 seems to be useful as a compromise from the viewpoint of regularity properties of the basis function $\psi(t)$ (Dremin *et al* 2001). Let us discuss whether the discussed inter-group differences can be improved by using other wavelet functions. As it was already discussed in section 2.5, the multiresolution analysis can be interpreted as the mathematical microscope that needs to be appropriately adjusted to reveal necessary details, e.g., to illuminate small changes in the structure of experimental time series at early stages of the transition from normal to pathological dynamics. Figure 7 testifies that the selection of the wavelet-basis is an important issue for data processing.

Following figure 7, good separations between the characteristics of the considered groups are obtained also for the Daubechies wavelets D^4 (symmetric function ψ) and D^{12}. However, we do not improve the diagnostic abilities of the multiresolution analysis by changing the

used basis. Let us note that some wavelet functions are not appropriate for the analysis of drug effects. Thus, the non-symmetric wavelet D^8 was unable to distinguish between healthy and pathological dynamics. Using the analogy between the multiresolution analysis and a 'mathematical microscope' we can conclude about the selection of an objective that does not allow the revealing of necessary details. 'Good objectives' represented by the functions D^4, D^8 (symmetric wavelet) and D^{12} demonstrate quite similar results. This means that if the wavelet is chosen appropriately, other 'objectives' do not provide new information (Dremin *et al* 2001, Pavlov and Anishchenko 2007).

4. Discussion

In this study we analyzed pathological changes in cerebral venous hemodynamics at different stages of ICH in newborn mice and the role of adrenodependent vasorelaxation mechanisms in this process using wavelet analysis of vascular dynamics of the sagittal sinus. To induce ICH in newborn mice we used the harmful effects of infrasound with high intensity on the brain. This severe stress causes functional disorder and structure injury of the central nervous system, especially in the cortex. The superficial small multiple ICH is the most frequently occurring cerebral disorder induced by infrasound due to its deleterious effects on the vegetovascular centers. In our previous works, we obtained the multiple ICH in the cerebral cortex in adult and newborn rats subjected to the influence of infrasound (Semyachkina-Glushkovskaya *et al* 2013a, 2013b). Recent clinical studies showed that term neonates have predominantly superficial ICH in the cortex and subdural space (Gupta *et al* 2009, Whitby *et al* 2004, Looney *et al* 2007).

Although the mechanisms underlying ICH in neonates remain unknown, there is evidence that the critical pathological changes in CBF are the main reason. Thus, a correction of cerebral blood flow after ICH is the first-line therapy in newborns (Azhan and Wong 2012, Di Gennaro *et al* 2011, Ballabh 2010). Fluctuating CVBF associated with abnormalities of the venous blood flow is an important factor of the disturbance of cerebral hemodynamics associated with ICH (Volpe 2008, Gupta *et al* 2009). It is crucial, however, that there is limited information regarding the prognostic criteria for pathological changes in the pattern of CVBF. This is one of the reasons of difficulties in the determination of the true incidence of ICH (Gupta *et al* 2009, Lo 2011). Taking into consideration these circumstances, we studied the alterations of cerebral venous circulation at different stages of ICH—4 h after the infrasound influences (the transient stage without ICH) and 24 h after sound stress (the incidence of ICH).

The obtained results show that the development of ICH in newborn mice is accompanied by significant changes in the cerebral venous circulation and by differences between the standard deviations (σ) of the wavelet-coefficients d_j corresponding to the states before and after stress. The transient stage of ICH is accompanied by an increased diameter of the sagittal sinus with the fall of the velocity of blood flow. The incidence of ICH is characterized by more pronounced dilation of the sagittal sinus with the decreased velocity of blood flow. These changes are associated with the increased variance of wavelet-coefficients at the levels of resolution $j=[3;7]$ that include the area of very slow frequencies ($j\leqslant 6$) and the range of slow dynamics ($j\geqslant 6$) figure 4. Because LSCI-data was recorded with the sample step 1 s, we do not consider larger j associated with higher frequencies (thus, $j\geqslant 8$ corresponds to frequencies >1 Hz). Analyzing different values of j we can, therefore, reveal the effects of different mechanisms influenced by the dynamics of CVBF.

The dilation of intracranial veins and the engorgement of venous sinuses are the symptoms of elevation of the cerebral venous pressure and the cerebral hypotension that are

associated with ICH in newborns (Ghazi-Birry *et al* 1997, Mokri 2004, Volpe 2008). We suggest that the relaxation of the sagittal sinus with the decreased velocity of blood flow precedes to ICH in newborn mice and can be considered as the prognostic criteria for a risk of ICH during the first days after the birth. Similar observations were reported in newborn rats (Semyachkina-Glushkovskaya *et al* 2013a). The obtained experimental results are in good accordance with clinical data suggesting that the decrease of the cerebral venous blood outflow is implicated in contributing to the occurrence of ICH in newborns (Ment *et al* 1984, Meek *et al* 1999). It was also reported that the impairment of CVBF with low cerebral perfusion pressure is associated with ICH and poor outcomes after brain injury in children (Munro *et al* 2004). Pediatric Guidelines recommend preventing venous insufficiency to avoid ICH (Adelson *et al* 2012).

A high activation of the adrenodependent vasorelaxation mechanism can represent one of the reasons underlying the critical dilation of the cerebral veins associated with ICH. Adrenergic-related vasorelaxation effects are realized by the activation of β_2-adrenoreceptors (Lands *et al* 1967, Wilffert *et al* 1982) with increased activity of K_{ATP}-channels of the vascular muscle (Nakashima and Vanhoutte 1995, Wang and Lipsius 1995, Koumi *et al* 1995, Fujii *et al* 1999) and the production of endothelial nitric oxide (Dawes *et al* 1997, Uchida *et al* 2002, Ferro *et al* 2004).

Based on these results we studied the effects of the pharmacological stimulation of β_2-adrenoreceptor-mediated vasorelaxation with isoproterenol (agonist of β_2-adrenoreceptors) on the cerebral venous hemodynamics. This study was performed in newborn mice under the normal state and at different stages of ICH with an evaluation of changes in endothelial and non-endothelial components of CVBF-variability.

We revealed the highest sensitivity of the sagittal sinus to isoproterenol in animals is at the transient stage of ICH. Indeed, isoproterenol-induced increase in the diameter of the sagittal sinus was two-fold higher while the corresponding decrease in CVBF was one, five-fold greater in newborn mice at 4 h after the stress compared with healthy animals. These changes of CVBF in the masked period of ICH are accompanied by differences in wavelet-coefficients between healthy and sick mice: the clearest group separation was observed for $j=3$ that is associated with high activation of endothelial factors of the vascular tone regulation figure 5. In general, we revealed inter-group differences for $j=[3, 6]$. These facts are consistent with experimental data suggesting that β_2-adrenomediated vasorelaxation is realized through stimulation of both, endothelial (NO-system) and non-endothelial (K_{ATP}-channels activity of vascular muscle) mechanisms (Nakashima and Vanhoutte 1995, Wang and Lipsius 1995, Fujii *et al* 1999, Dawes *et al* 1997, Uchida *et al* 2002, Ferro *et al* 2004).

The development of ICH is accompanied by the progression of pathological changes in CVBF associated with more pronounced differences of variability of wavelet-coefficients compared with mice at a masked period of ICH and, especially, with healthy mice figure 6. In mice with ICH, the diameter of the sagittal sinus was maximal compared with normal and transient states. Isoproterenol did not cause the additional relaxation of the dilated venous vessel, however the velocity of CVBF decreased.

Using isoproterenol we modulated critical changes in CVBF by stimulation of additional adrenorelated vasorelaxation in mice with ICH characterized by the dilated cerebral veins. Despite the absence of clear changes in physiological parameters of CVBF (such as the diameter of the sagittal sinus and the velocity of CVBF) in mice with ICH after isoproterenol injection, multiresolution analysis reveals significant changes in the dynamics of CVBF quantifying with the variability of wavelet-coefficients. This allows us to conclude that the multiresolution analysis of cerebrovascular dynamics represents a useful tool for the evaluation of critical changes in CVBF associated with a risk of ICH.

It is known that vasodilatory capabilities of immature cerebral vessels are significantly limited (Cavazzutti and Duffy 1982). Thus, vasodilatory effects of dopamine do not appear in preterm fetal lambs with severe hypoxia that is accompanied by the dilation of cerebral vessels (Mayock *et al* 2007). These facts partly explain why a severely dilated sagittal sinus was insensitive to isoproterenol in newborn mice with ICH. Higher sensitivity of the sagittal sinus to isoproterenol in stressed newborn mice at the transient stage of ICH can be related to a higher binding affinity of non-innervated β_2-adrenoreceptors to the circulating adrenaline—the main hormone of stress releasing from the adrenal glands (Wilffert *et al* 1982, Broadley *et al* 1986). Some early experimental studies of newborn rats showed that the adrenergic synaptic fibers in the brain develop slowly; they reach adult levels approximately two months after the birth (Harden *et al* 1977, Brown and Goldman 1977). Intensive maturation of the vascular β_2-adrenoreceptors occurs more rapidly compared with adrenergic synapses—during the first two weeks after the birth (Harden *et al* 1977). Thus, high density and sensitivity of non-innervated vascular β_2-adrenoreceptors in newborn mice can be one of the key reasons for the relaxation of the cerebral vessels under the stress condition. These facts allow us to assume that the predominance of adrenergic-related vasodilatory responses to stress in newborn mice is an important mechanism underlying the development of ICH during the first days after the birth. Further studies are needed to elucidate the particularities of molecular mechanisms by which β_2-adrenergic receptors regulating cerebral circulation, trigger critical changes in CBF, thus leading to the ICH in newborns.

5. Conclusion

In summary, we showed that the dilation of the sagittal sinus with the decreased velocity of blood flow presides to the stress-induced ICH in newborn mice. These morphofunctional vascular changes are associated with a pronounced increase in the variance of wavelet-coefficients in the area of endothelial and non-endothelial (sympathetic) components of CVBF variability.

The revealed changes in the cerebral venous hemodynamics at the latent stage of ICH are accompanied by high responsiveness of the sagittal sinus to isoproterenol that are reflected in the statistics of wavelet-coefficients related to the region of very slow frequencies that correspond to endothelial (NO) and non-endothelial (K_{ATP}-channels activity of vascular muscle) sympathetic mechanisms of isoproterenol. The indicated changes in the variability of CVBF induced by isoproterenol are manifested more clearly in mice with ICH. These facts certify a higher activation of the adrenergic-related vasodilatory responses to severe stress in newborn mice that can be one of the important mechanisms underlying the development of ICH.

Thus, the venous insufficiency with the decreased blood outflow from the brain associated with changes in the endothelial and the sympathetic components of CVBF variability can be treated as prognostic criteria for a risk of ICH during the first days after birth.

Acknowledgments

The authors thank O V Ivanov for providing us with high-precision (64 digits) filters coefficients. This work was supported by the grants of the Science Fund for Creative Research Group of China (Grant No. 61121004), the National Nature Science Foundation of China (Nos. 81171376, 91232710), the grant NSFC-RFBR for International Cooperation (No. 812111313) and the Russian Foundation for Basic Research (Grant No. 14-02-00526-a). AP acknowledges support from the RF Ministry of Education and Sciences within the project 3.23.2014/K (grant SGTU-157). OS-G and VT acknowledge support from the Mega grant No. 14.Z50.31.0004 and OS-G and

OB acknowledge support from the RF Ministry of Education and Sciences within the project 17.488.2014/K and the RF President program for young scientists (14.Z56.14.2216-MD). VT acknowledges support from grants FiDiPro, TEKES Program (40111/11), Finland, SCOPES EC, Uzb/Switz/RF, Swiss NSF, IZ74ZO_137423/1 and Russian Presidential grant NSH-703.2014.2.

References

Adelson P D *et al* 2012 Guidelines for the acute medical management of severe traumatic brain injury in infants, children, and adolescents *Pediatr. Crit. Care Med.* **13** S1

Addison P S 2002 *The Illustrated Wavelet Transform Handbook: Applications in Science, Engineering, Medicine and Finance* (Philadelphia: IOP Publishing)

Azhan A and Wong F 2012 Challenges in understanding the impact of blood pressure management on cerebral oxygenation in the preterm brain *Front Physiol.* **3** 471

Ballabh P 2010 Intraventricular hemorrhage in premature infants: mechanism of disease *Pediatr. Res.* **67** 1

Bevan J A, Bevan R D and Duckles S P 1981 Adrenergic regulation of vascular smooth muscle *The Handbook of Physiology. The Cardiovascular System. Vascular Smooth Muscle* vol II (Bethesda: American Physiological Society) p 515

Boas D A and Dunn A K 2010 Laser speckle contrast imaging in biomedical optics *J. Biomed. Opt.* **15** 011109

Bray R C, Forrester K R, Reed J, Leonard C and Tulip J 2006 Endoscopic laser speckle imaging of tissue blood flow: applications in the human knee *J. Orthop. Res.* **24** 1650

Broadley K J, Chess-Williams R G and Grassby P F 1986 A physiological basis for subclassifying beta-adrenoceptors examined by chemical sympathectomy of guinea-pigs *J. Physiol.* **373** 367

Brown R M and Goldman P S 1977 Catecholamines in neocortex of rhesus monkeys: regional distribution and ontogenetic development *Brain Res.* **124** 576

Briers J D and Fercher A F 1982 Retinal blood-flow visualization by means of laser speckle photography *Invest. Ophthalmol. Vis. Sci.* **22** 255

Briers J D and Webster S 1996 Laser speckle contrast analysis (LASCA): a non-scanning, full-field technique for monitoring capillary blood flow *J. Biomed. Opt.* **1** 174

Briers J D 2001 Laser Doppler, speckle and related techniques for blood perfusion mapping and imaging *Physiol. Meas.* **22** R35

Busija D W and Heistad D D 1984 Factors involved in the physiological regulation of cerebral circulation *Rev. Physiol. Biochem. Pharmacol.* **101** 161

Cavazzutti M and Duffy T E 1982 Regulation of local cerebral blood flow in normal and hypoxic newborn dogs *Ann. Neurol.* **1** 247

Daubechies I 1992 *Ten Lectures on Wavelets* (Philadelphia: Society for Industrial and Applied Mathematics)

Dawes M, Chowienczyk P J and Ritter J M 1997 Effects of inhibition of the L-arginine/nitric oxide pathway on vasodilation caused by β-adrenergic agonists in human forearm *Circulation* **95** 2293

Di Gennaro J L, Mack C D, Malakouti A, Zimmerman J J, Armstead W and Vavilala M S 2010 Use and effect of vasopressors after pediatric traumatic brain injury *Dev. Neurosci.* **32** 420

Dremin I M, Ivanov O V and Nechitailo V A 2001 Wavelets and their uses *Phys.–Usp.* **44** 447

Edvinsson L and Hamel E 2002 Perivascular nerves in brain vessels *Cerebral Blood Flow and Metabolism* (Philadelphia: Williams & Wilkins)

Edvinsson L, MacKenzie E T and McCulloch J 1993 Adrenergic mechanisms *Cerebral Blood Flow and Metabolism* (New York: Raven Press)

Ferro A, Coash M, Yamamoto T, Rob J, Ji Y and Queen L 2004 Nitric oxide-dependent beta2-adrenergic dilatation of rat aorta is mediated through activation of both protein kinase A and Akt *Br. J. Pharmacol.* **143** 397

Forrester K R, Stewart C, Tulip J, Leonard C and Bray R C 2002 Comparison of laser speckle and laser Doppler perfusion imaging: measurement in human skin and rabbit articular tissue *Med. Biol. Eng. Comput.* **40** 687

Forrester K R, Tulip J, Leonard C, Stewart C and Bray R C 2004 A laser speckle imaging technique for measuring tissue perfusion *IEEE Trans. Biomed. Eng.* **51** 2074

Fujii K, Onaka U, Goto K, Abe I and Fujishima M 1999 Impaired isoproterenol-induced hyperpolarization in isolated mesenteric arteries of aged rats *Hypertension* **34** 222

Garland C J, Yarova P L, Jiménez-Altayó F and Dora K A 2011 Vascular hyperpolarization to β-adrenoceptor agonists evokes spreading dilatation in rat isolated mesenteric arteries *Br. J. Pharmacol.* **164** 913

Ghazi-Birry H S, Brown W R, Moody D M, Challa V R, Block S M and Reboussin D M 1997 Human germinal matrix: venous origin of hemorrhage and vascular characteristics *Am. J. Neuroradiol.* **18** 219

Gupta S N, Kechli A M and Kanamalla U S 2009 Intracranial hemorrhage in term newborn: management and outcomes *Pediatr. Neurol.* **40** 1

Hara M R *et al* 2011 A stress response pathway regulates DNA damage through β_2-adrenoreceptors and β-arrestin-1 *Nature* **477** 349

Harden T K, Wolfe B B, Sporn J R, Perkins J P and Molinoff P B 1977 Ontogeny of beta-adrenergic receptors in rat cerebral cortex *Brain Res.* **125** 99

Hare G M T, Worrall J M A, Baker A J, Liu E, Sikich N and Mazer C D 2006 β_2 adrenergic antagonist inhibits cerebral cortical oxygen delivery after severe haemodilution in rats *Br. J. Anaesth.* **97** 617

Koumi S, Wasserstrom J A and Ten Eick R E 1995 Beta-adrenergic and cholinergic modulation of inward rectifier K^+ channel function and phosphorylation in guinea-pig ventricle *J. Physiol.* **486** 661

Lands A M, Arnold A, McAuliff J P, Luduena F P and Brown T G 1967 Differentiation of receptor systems activated by sympathomimetic amines *Nature* **214** 597

Liu S, Li P and Luo Q 2008 Fast blood flow visualization of high-resolution laser speckle imaging data using graphics processing unit *Opt. Express* **16** 14321

Lo W D 2011 Childhood hemorrhagic stroke: an important but understudied problem *J. Child Neurol.* **26** 1174

Looney C B, Smith J K, Merck L H, Wolfe H M, Chescheir N C, Hamer R M and Gilmore J H 2007 Intracranial hemorrhage asymptomatic neonates: prevalence on MR images and relationship obstetric and neonatal risk factors *Radiology* **242** 535

Mayock D E, Bennett R, Robinson R D and Gleason C A 2007 Dopamine does not limit fetal cerebrovascular responses to hypoxia *J. Appl. Physiol.* **102** 130

McCalden T A 1981 Sympathetic control of the cerebral circulation *J. Auton. Pharmacol.* **1** 421

Meek J, Tyszczuk L, Elwell C and Wyatt J 1999 Low cerebral blood flow is a risk factor for severe intraventricular hemorrhage *Arch. Dis. Child Fetal Neonatal. Edn* **81** F15

Ment L R, Duncan C C, Ehrenkranz R A, Lange R C, Taylor K J, Kleinman C S, Scott D T, Sivo J and Gettner P 1984 Intraventricular hemorrhage in the preterm neonate: timing and cerebral blood flow changes *J. Pediatr.* **104** 419

Meyer Y 1993a *Wavelets: Algorithms and Applications* (Philadelphia: Society for Industrial and Applied Mathematics)

Meyer Y 1993b *Wavelets and Operators* (Cambridge: Cambridge University Press)

Mokri B 2004 Spontaneous low cerebrospinal pressure/volume headaches *Curr. Neurol. Neurosci. Rep.* **4** 117

Munro M J, Walker A M and Barfield C P 2004 Hypotensive extremely low birth weight infants have reduced cerebral blood flow *Pediatrics* **114** 1591

Nakashima M and Vanhoutte P M 1995 Isoproterenol causes hyperpolarization through opening of ATP-sensitive potassium channels in vascular smooth muscle of the canine saphenous vein *J. Pharmacol. Exp. Ther.* **272** 379

Pavlov A N and Anishchenko V S 2007 Multifractal analysis of complex signals *Phys.—Usp.* **50** 819

Pavlov A N, Nazimov A I, Pavlova O N, Lychagov V V, Tuchin V V, Bibikova O A, Sindeev S S and Semyachkina-Glushkovskaya O V 2014 Wavelet-based analysis of cerebrovascular dynamics in newborn rats with intracranial hemorrhages *J. Innov. Opt. Health Sci.* **7** 1350055

Pavlov A N, Semyachkina-Glushkovskaya O V, Pavlova O N, Bibikova O A and Kurths J 2013 Wavelet-analysis of gastric microcirculation in rats with ulcer bleedings *Eur. Phys. J. Spec. Top.* **222** 2705

Perlman J M, McMenamin J B and Volpe J J 1983 Fluctuating cerebral blood-flow velocity in respiratory-distress syndrome. Relation to the development of intraventricular hemorrhage *New Engl. J. Med.* **309** 204

Semyachkina-Glushkovskaya O *et al* 2013a The assessment of pathological changes in cerebral blood flow in hypertensive rats with stress-induced intracranial hemorrhage using Doppler OCT: particularities of arterial and venous alterations *J. Photon. Lasers Med.* **2** 109

Semyachkina-Glushkovskaya O V *et al* 2013b The experimental study of stress-related pathological changes in cerebral venous blood flow in newborn rats assessed by DOCT *J. Innov. Opt. Health Sci.* **6** 1350023

Stauss H M 2007a Power spectral analysis in mice: What are the appropriate frequency bands? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **292** R902

Stauss H M 2007b Identification of blood pressure control mechanisms by power spectral analysis *Clin. Exp. Pharmacol. Physiol.* **34** 362

Stewart C J, Gallant-Behm C L, Forrester K R, Tulip J, Hart D A and Bray R C 2006 Kinetics of blood flow during healing of excisional full-thickness skin wounds in pigs as monitored by laser speckle perfusion imaging *Skin Res. Technol.* **12** 247

Suzuki Y, Masuda K, Ogino K, Sugita T, Aizu Y and Asakura T 1991 Measurement of blood flow velocity in retinal vessels utilizing laser speckle phenomenon *Japan. J. Ophthalmol.* **35** 4

Thurner S, Feurstein M C and Teich M C 1998 Multiresolution wavelet analysis of heartbeat intervals discriminates healthy patients from those with cardiac pathology *Phys. Rev. Lett.* **80** 1544

Tuchin V V 2013 Handbook of Coherent-Domain Optical Methods: Biomedical Diagnostics, Environmental Monitoring, and Materials Science 2nd edn (New York: Springer)

Uchida S, Kawashima K and Lee T J 2002 Nicotine-induced NO-mediated increase in cortical cerebral blood flow is blocked by beta2-adrenoceptor antagonists in the anaesthetized rats *Auton. Neurosci.* **96** 126

Volpe J J 2008 Intracranial hemorrhage: germinal matrix-intraventricular hemorrhage *Neurology of the Newborn* (Philadelphia: Saunders) p 517

Wang Y G and Lipsius S L 1995 β-adrenergic stimulation induces acetylcholine to activate ATP-sensitive K^+ current in cat arterial myocytes *Circ. Res.* **77** 565

Whitby E H, Grifths P D, Rutter S, Smith M F, Sprigg A, Ohadike P, Davies N P, Rigby A S and Paley M N 2004 Frequency and natural of subdural haemorrhages in babies and relation to obstetric factors *Lancet* **363** 846

Wilffert B, Timmermans P and van Zwieten P 1982 Extrasynaptic location of alpha-2 and non-innervated beta-2 adrenoreceptors in the vascular system of the pithed normotensive rat *J. Pharmacol. Exp. Ther.* **221** 762

Wong F Y, Leung T S, Austin T, Wilkinson M, Meek J H, Wyatt J S and Walker A M 2008 Impaired autoregulation in preterm infants identified by using spatially resolved spectroscopy *Pediatrics* **121** e604