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Diffusion properties of the brain in health and disease

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Abstract

Extrasynaptic transmission between neurons and communication between neurons and glia are mediated by the diffusion of neuroactive substances in the extracellular space (ECS)—volume transmission. Diffusion in the CNS is inhomogeneous and often not uniform in all directions (anisotropic). Ionic changes and amino acid release result in cellular (particularly glial) swelling, compensated for by ECS shrinkage and a decrease in the apparent diffusion coefficients of neuroactive substances or water (ADC_W). The diffusion parameters of the CNS in adult mammals (including humans), ECS volume fraction α (α = ECS volume/total tissue volume; normally 0.20–0.25) and tortuosity λ ($\lambda^2 = D/\mathrm{ADC}$; normally 1.5–1.6), hinder the diffusion of neuroactive substances and water. A significant decrease in ECS volume and an increase in diffusion barriers (tortuosity) and anisoptropy have been observed during stimulation, lactation or learning deficits during aging, due to structural changes such as astrogliosis, the re-arrangement of astrocytic processes and a loss of extracellular matrix. Decreases in the apparent diffusion coefficient of tetramethylammonium (ADC_{TMA}) and ADC_W due to astrogliosis and increased proteoglycan expression were found in the brain after injury and in grafts of fetal tissue. Tenascin-R and tenascin C-deficient mice also showed significant changes in ADC_{TMA} and ADC_W, suggesting an important role for extracellular matrix molecules in ECS diffusion. Changes in ECS volume, tortuosity and anisotropy significantly affect neuron-glia communication, the spatial relation of glial processes towards synapses, the efficacy of glutamate or GABA 'spillover' and synaptic crosstalk, the migration of cells, the action of hormones and the toxic effects of neuroactive substances and can be important for diagnosis, drug delivery and new treatment strategies.

Keywords: Anisotropy; Apparent diffusion coefficient; Extracellular matrix; Diffusion-weighted NMR; Tortuosity; Volume fraction

1. Introduction

The efficacy of diffusion of many neuroactive substances in the CNS extracellular space (ECS) is dependent on the size, electrical charge, shape and structure of the respective substance, as well as on the physico-chemical properties of the ECS. ECS diffusion properties differ with the anatomical structure of a particular brain region, during CNS development, neural activity, hormonal release (e.g. during lactation), aging, and in many pathological states (Nicholson and Syková, 1998; Syková and Chvátal, 2000; Syková et al., 2000; Syková, 2001a, 2002).

Neurons interact both by synapses and by the diffusion of neuroactive substances in the extracellular space (ECS). Glial cells do not have synapses and therefore, communication with neurons is achieved only by the diffusion of ions and neuroactive substances in the ECS. Even the interaction of a neuron with glial cells at a single synapse must therefore involve the diffusion of a transmitter and ions released by the neuron to the synaptic cleft via the nearby ECS (Fig. 1). Both neurons and glia release ions, transmitters and various other neuroactive substances that diffuse beyond a single synpase, to greater distances from the release site (Fig. 1). Substances diffuse through the ECS and bind to extrasynaptic, usually high-affinity, binding sites located on neurons, axons and glial cells. This type of nonsynaptic transmission is also called extrasynaptic transmission or volume transmission (neuroactive substances move through the volume of the ECS) (Fuxe and Agnati, 1991; Agnati et al., 1995; Syková, 1997; Nicholson and Syková, 1998; Zoli et al., 1999). Synaptic communication is typically one to one communication, while volume transmission is communication of one to many others. This mode of communication without synapses provides a possible mechanism for synchronizing neuronal activity and long-range information processing in functions such as vigilance, sleep, lactation, chronic pain, hunger, depression, LTP, LTD, memory formation and other plastic changes in the CNS (Syková, 1997).

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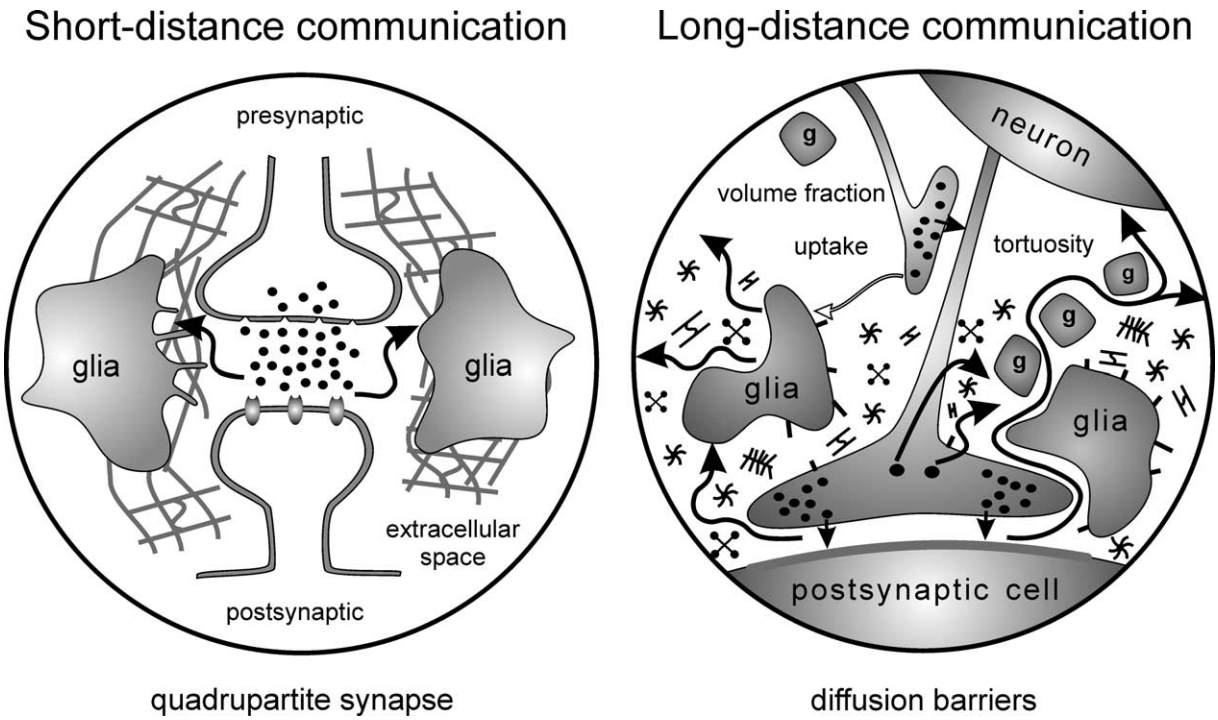

Fig. 1. Closed synapses are typical of synaptic transmission. These synapses are often ensheathed by glial processes and by the extracellular matrix, forming perineuronal or perisynaptic nets. The extracellular space (ECS) changes its diffusion parameters in response to neuronal activity and glial cell re-arrangement. In short-distance communication by diffusion, presynaptic terminals, postsynaptic terminals, glial cell processes and the ECS form a "plastic" quadrupartite synapse. Long-distance communication: The CNS architecture is composed of neurons (N), axons, glial cells (glia), cellular processes (G), molecules of the extracellular matrix and intercellular channels between the cells. This architecture slows down the movement (diffusion) of substances in the brain, which is critically dependent on the ECS diffusion parameters volume fraction (α), tortuosity (λ) and nonspecific uptake (k').

The diffusion properties of the ECS vary around each cell and in different brain regions. Certain synapses ("private synapses") or even whole neurons are clearly tightly ensheathed by glial processes and by the extracellular matrix, so-called perineuronal nets (Celio et al., 1998); others are left more "naked". Many synapses, together with glial processes, form a complex that can be called a "quadrupartite" synapse, which is formed by a presynpatic terminal of a neuron, a postsynaptic cell membrane, an astrocyte process and the ECS (Fig. 1). In fact, transmitters such as glutamate or GABA can escape from the synaptic cleft (especially during repetitive stimulation and/or the massive release of a transmitter) and affect extrasynaptic receptors on glia or neurons or reach a neighboring synapse. These phenomena have been called "transmitter spillover" and "synaptic cross-talk" (Kullmann et al., 1996; Asztely et al., 1997). Recently, we found that the size of the ECS differs around certain cell types, e.g. it is smaller around oligodendrocytes than around astrocytes (Chvátal et al., 1999; Vargová et al., 2001), or during structural re-arrangement of glial processes, e.g. during development, lactation or aging (Lehmenkühler et al., 1993; Syková et al., 1998, 2002; Vargová et al., 2002).

The size and irregular geometry of diffusion channels in the ECS (tissue tortuosity and anisotropy) substantially differ not only around cells but particularly in various CNS regions. The diffusion parameters thereby modulate neuronal signaling, neuron-glia communication and extrasynaptic transmission. Besides their changes during brain development, glial cell maturation, lactation and aging, diffusion parameters are changed due to cell proliferation, hypertrophy, cell swelling and neuronal loss. They are substantially altered during CNS injury, anoxia/ischemia, spreading depression, epileptic seizures, inflammation and demyelination, in grafted tissue and tumors, and during many other brain pathological states (Syková et al., 2000; Syková and Chvátal, 2000; Syková, 1997, 2001a, 2002).

1.1. ECS diffusion parameters: volume fraction and tortuosity

The diffusion of substances in a free medium is described by Fick's laws. In contrast to free medium, diffusion in the ECS of the nervous tissue is hindered by the size of the extracellular clefts, the presence of membranes, fine neuronal and glial processes, macromolecules of the extracellular matrix, adhesion molecules, charged molecules and also by cellular uptake (Fig. 1). To take into account these factors, it was necessary to modify Fick's original diffusion equations (Nicholson and Phillips, 1981; Nicholson and Syková, 1998). First, diffusion in the CNS is constrained by the restricted volume of the tissue available for diffusing particles,

i.e. by the ECS volume fraction (α), which is a dimensionless quantity and is defined as the ratio between the volume of the ECS and the total volume of the tissue. It is now evident that the ECS in adult brain amounts to about 20% of the total brain volume, i.e. $\alpha = 0.2$. Second, the free diffusion coefficient (D) in the brain is reduced by the tortuosity factor (λ). ECS tortuosity is defined as $\lambda = (D/\mathrm{ADC})^{0.5}$, where D is a free diffusion coefficient and ADC is the apparent diffusion coefficient in the brain. As a result of tortuosity, D is reduced to an apparent diffusion coefficient $\mathrm{ADC} = D/\lambda^2$. Thus, any movement of a substance diffusing in the ECS is hindered by a number of obstacles (diffusion barriers) (Fig. 1). Third, substances released into the ECS are transported across membranes by non-specific concentration-dependent uptake (k'). In many cases however, substances are transported by energy-dependent uptake systems that obey non-linear kinetics (Nicholson, 2001). When these three factors (α, λ and k') are incorporated into Fick's laws, diffusion in the ECS is described fairly satisfactorily (Nicholson and Phillips, 1981).

1.2. The methods used to study ECS diffusion parameters

The real-time iontophoretic method is the only method that can be used to determine the absolute values of all three ECS diffusion parameters (α, λ and k') and their dynamic changes in nervous tissue in vivo as well as in vitro (Syková, 1997; Nicholson and Syková, 1998). Ion-selective microelectrodes (ISM) are used to measure the diffusion of ions for which cell membranes are relatively impermeable, e.g. tetraethylammonium (TEA^+), tetramethylammonium (TMA^+), choline, or anions such as AsF_6 or α-naphthalene sulphonate (Nicholson and Phillips, 1981). These substances are administered into the nervous tissue by pressure injection or by an iontophoretic electrode, which is aligned parallel to a double-barreled ISM at a fixed distance (Fig. 2A). Usually, such an electrode array is made by gluing together a pressure or iontophoretic pipette and an ISM with a tip separation of 100–200 μm. In the case of iontophoretic application, TMA^+ is released into the ECS by applying a current step of +80 nA with a duration of 40–80 s. The released TMA^+ is recorded with the TMA^+-ISM as a diffusion curve, which is then transferred to a computer. The values of ECS volume, ADC, tortuosity and non-specific cellular uptake are extracted by a non-linear curve-fitting simplex algorithm operating on the diffusion curve described by Eq. (1) below, which represents the behavior of TMA^+, assuming that it spreads out with spherical symmetry, when the iontophoresis current is applied for duration S. In this expression, C is the concentration of the ion at time t and distance r. The equation governing diffusion in brain tissue is:

$$C = G(t) \quad t < S, \qquad \text{for the rising phase of the curve}$$

$$C = G(t) - G(t-S) \quad t > S, \qquad \text{for the falling phase of the curve.}$$

The function $G(u)$ is evaluated by substituting t or $t - S$ for u in the following equation (Rice et al., 1993):

$$G(u) = \left(\frac{Q\lambda^2}{8\pi D\alpha r}\right)\left\{\exp\left[r\lambda\left(\frac{k'}{D}\right)^{1/2}\right] \times \mathrm{erfc}\left[\frac{r\lambda}{2(Du)^{1/2}} + (k'u)^{1/2}\right] + \exp\left[-r\lambda\left(\frac{k'}{D}\right)^{1/2}\right] \times \mathrm{erfc}\left[\frac{r\lambda}{2(Du)^{1/2}} - (k'u)^{1/2}\right]\right\} \quad (1)$$

The quantity of TMA^+ delivered to the tissue per second is $Q = In/zF$, where I is the step increase in current applied to the iontophoresis electrode, n the transport number, z the number of charges associated with the substance iontophoresed (+1 for TMA^+ or TEA^+), and F Faraday's electrochemical equivalent. The function "erfc" is the complementary error function. When the experimental medium is agar, by definition, $\alpha = 1 = \lambda$ and $k' = 0$, and the parameters n and D are extracted by the curve fitting. Knowing n and D, the parameters α, λ and k' can be obtained when the experiment is repeated in neural tissue.

The other methods used so far to study ECS volume and geometry in vivo have been less comprehensive, since either they cannot provide information about all three diffusion parameters or they only give relative values. These methods include measurements of tissue resistance (Van Harreveld et al., 1971; Matsuoka and Hossmann, 1982; Korf et al., 1988), measurements of intrinsic optical signals (IOS) (Syková et al., 2003), measurements of the ADCs of molecules tagged with fluorescent dye, so-called integrative optical imaging (IOI) (Prokopová-Kubinová et al., 2001; Nicholson and Tao, 1993) or diffusion-weighted NMR imaging (DW-MRI). In many of our studies, we compared two methods (the TMA^+ method and IOS, IOI or DW-MRI) in physiological states as well as in animal models of pathological states, and moreover we related the data to morphological changes determined by immunohistochemical methods. In vivo, changes in ECS diffusion parameters were studied during development, aging, lactation, inflammation, demyelination, Parkinson's disease, hydrocephalus, during or after anoxia/ischemia, after CNS injury and in various knock-out animals to mimic pathological states.

Diffusion-weighted NMR methods (Benveniste et al., 1992; Latour et al., 1994; Norris et al., 1994; Van der Toorn et al., 1996) give information about the diffusion coefficient of water, but the relationship between water movement, ADC_W maps and changes in ECS volume fraction and tortuosity due to cell swelling and structural changes is not yet well understood. Fig. 3 shows clearly that water diffusion in the rat brain is inhomogeneous (ADC_W maps). We can clearly identify different brain regions that correspond to those recognized histologically (Fig. 3: GFAP and

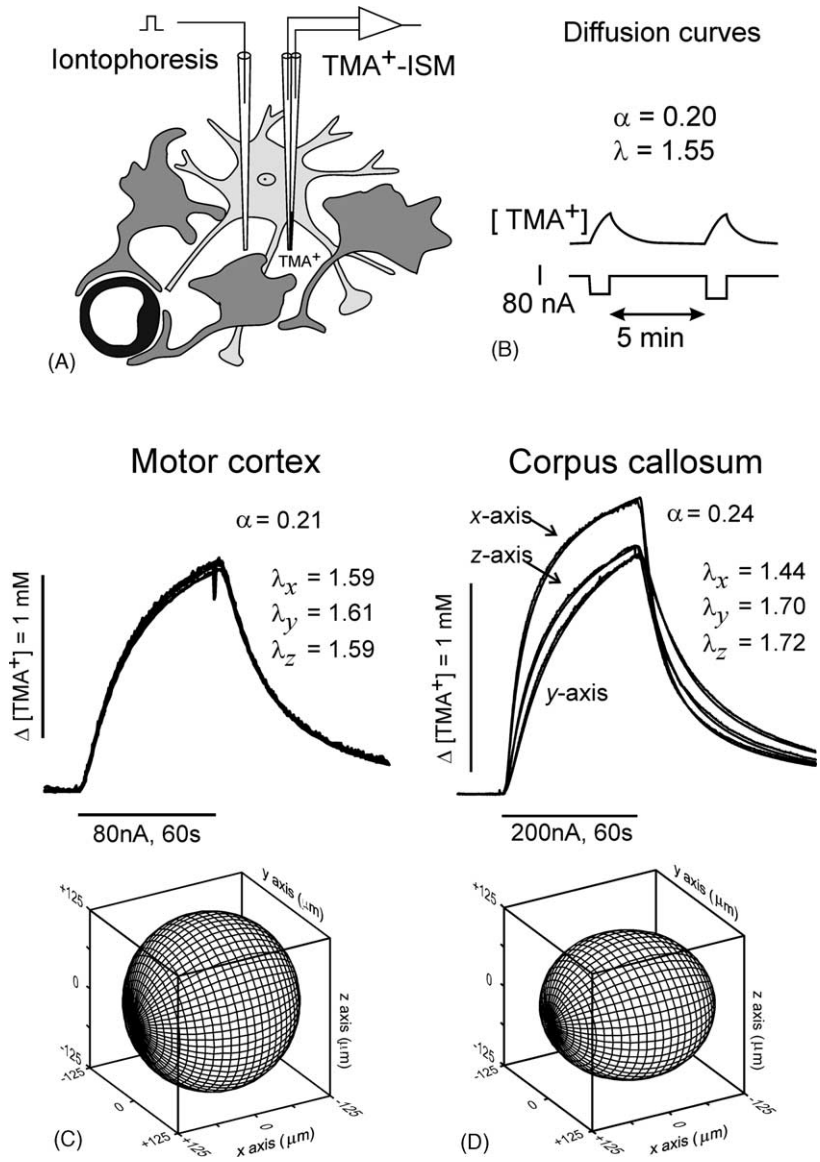

Fig. 2. Measurements of extracellular space diffusion parameters. (A) A TMA^+-selective double-barreled microelectrode (TMA^+-ISM) is glued to a TMA^+ iontophoresis microelectrode with a known inter-tip distance, and this array is introduced into the tissue. (B) TMA^+ diffusion curves and typical ECS diffusion parameters volume fraction (α) and tortuosity (λ) obtained in the brain after the application of an iontophoretic current of 80 nA. The ECS in normal brain is 20% ($\alpha = 0.20$) and tortuosity is 1.55. (C and D) Representative diffusion curves obtained in the motor cortex and corpus callosum in the *x*-axis (along the axons) and the *y*- and *z*-axes (across the axons). All recordings are from same animal. The values of α and λ are shown with each curve. The differences in the shape and amplitude of the diffusion curves reflect the different tortuosity values found in different directions, so-called diffusion anisotropy. The diffusion spheres for the motor cortex and corpus callosum, representing isoconcentration surfaces for a 1 mM TMA^+ concentration contour 10 s after the onset of a 200 nA iontophoretic pulse, reveal isotropic diffusion in cortical gray matter and anisotropic diffusion in the corpus callosum (ellipsoidal surface).

CSPG); with sufficient resolution we can even recognize on ADC_W maps the different hippocampal layers. Comparing the TMA^+ method with DW-MRI, we have found that decreases in ADC_W can be related either to decreases in ECS volume fraction (Van der Toorn et al., 1996) or to increases in tortuosity without apparent changes in ECS volume (Voříšek et al., 2002) (Fig. 3) or to changes in both diffusion parameters.

Recordings of intrinsic optical signals (IOS), either light transmittance or light reflectance, are believed to reflect changes in the ECS volume; however, the evidence is not compelling. Recently, it became evident that direct evidence is missing, since under a number of experimental conditions the relationship does not hold (Syková et al., 2003; Jarvis et al., 1999; Tao et al., 2002). We measured light transmittance, ECS volume fraction (α) and extracellular K^+ in rat spinal cord slices during electrical stimulation and the application of elevated potassium, NMDA or anisoosmotic solutions. Dorsal root stimulation (10 Hz/1 min) induced an elevation in extracellular K^+ to 6–8 mM, a light

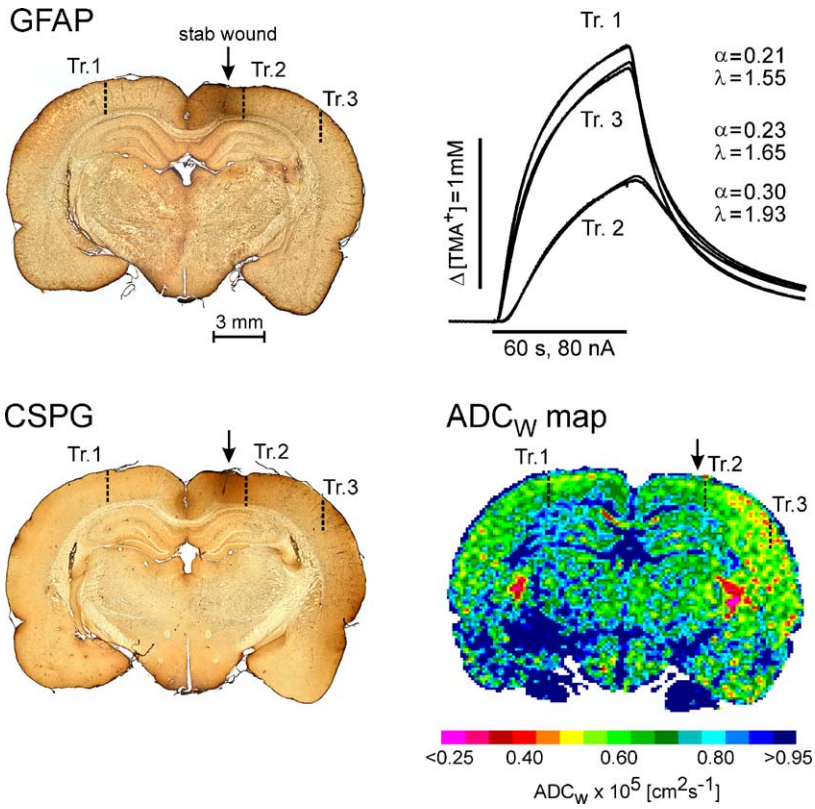

Fig. 3. Coronal rat brain sections stained for GFAP (glial fibrillary acidic protein—upper left) and CSPG (chondroitin-sulfate proteoglycan—lower left) 7 days following a cortical stab wound. The results show a higher level of GFAP expression in the vicinity of the stab wound and a higher level of CSPG expression in the whole cortex of the injured hemisphere. Both the GFAP and CSPG sections are from the same animal. The arrows indicate the site of the stab wound. Diffusion curves were recorded in 3 microelectrode tracks (Tr. 1, 2 and 3) using the TMA^+ method (upper right). Typical examples of TMA^+ diffusion curves and the corresponding values of volume fraction (α) and tortuosity (λ) obtained in the contralateral (Tr. 1) and wounded hemispheres of the same animal at distances of about 300 μm (Tr. 2) and 3000 μm (Tr. 3) from the stab wound at a depth of 1100 μm. The concentration scale is linear, and the theoretical diffusion curve is superimposed on each data curve. The pseudocolor image (lower right) shows a typical ADC_W map of an injured rat brain 7 days post-wounding; ADC_W was measured along the *y*-axis (rostro-caudal plane). The scale below the ADC_W map shows the relation between the intervals of ADC_W values and the colors used for visualization. ADC_W at 7 days post-wounding is significantly lower in the entire cortex of the wounded hemisphere compared to control animals, except in the area close to the wound. Positions where the diffusion curves were measured using the TMA^+ method are marked as Tr. 1–3.

transmittance increase of 6–8% and a relative ECS volume decrease of less than 5%, which is unlikely to be biologically significant; all of these changes had different time courses. The application of 6 or 10 mM K^+ or NMDA (10^{-5} M) had no measurable effect on ECS volume, but light transmittance increased by 20–25%. The application of 50 or 80 mM K^+ evoked a 72% decrease in α while the light transmittance increase remained as large as that during the application of 6 or 10 mM K^+. While the change in α persisted throughout the 45 min application, light transmittance, after peaking in 6–8 min, quickly returned to control levels and decreased below them. Astrocytic hypertrophy was observed in 6, 10 and 50 mM K^+ (Syková et al., 2003). The same results followed the application of 10^{-4} M NMDA or hypotonic solution (160 mmol/kg). The elevation of extracellular K^+ after NMDA application, corresponding to increased neuronal activity, had a similar time course as the light transmittance changes. Furosemide, Cl^--free or Ca^{++}-free solutions blocked or slowed down the decreases in α, while the light transmittance increases were unaffected. In hypertonic solution (400 mmol/kg), α increased by 30–40%, while light transmittance decreased by 15–20%. Thus, there is considerable evidence that light transmittance changes do not correlate only with changes in ECS volume but are more strongly associated with neuronal activity and morphological changes in astrocytes (Syková et al., 2003).

1.3. Diffusion anisotropy

The ECS has a complicated and uneven geometry, which in a given structure depends on the type and number of cells, their processes, density and orientation, and the extracellular matrix. Diffusion is not uniform in all directions and is affected by the presence of diffusion barriers including neuronal and glial processes, myelin sheaths, macromolecules and molecules with fixed negative surface charges. This so-called anisotropic diffusion often preferentially channels the movement of substances as well as of water in the ECS in

one direction, (e.g. along axons in the corpus callosum) and may, therefore, be responsible for a certain degree of specificity in volume transmission. Using the TMA^+ method, diffusion anisotropy was found in the CNS in the molecular layer of the cerebellum (Rice et al., 1993), in the hippocampus (Mazel et al., 1998), and in the auditory but not in the somatosensory cortex (Syková et al., 1999), while other studies revealed that it is present in the myelinated white matter of the corpus callosum (Fig. 2C) or spinal cord white matter (Voříšek and Syková, 1997a; Chvátal et al., 1997; Prokopová et al., 1997). It was shown that diffusion anisotropy in white matter increases during development; initially, diffusion in the as-yet unmyelinated corpus callosum is isotropic, but it becomes more anisotropic as myelination proceeds (Voříšek and Syková, 1997a; Prokopová et al., 1997).

Glial cells maintain not only ECS ionic homeostasis, but also ECS volume homeostasis (by swelling and shrinking during ionic shifts); they produce various extracellular matrix molecules, and when hypertrophied or proliferating form diffusion barriers (Syková, 1997, 2001b, 2002). In this way, glial cells can critically affect diffusion anisotropy and tissue permissiveness.

1.4. Extracellular space diffusion parameters during development and demyelination

Compared to healthy adults, ECS diffusion parameters significantly differ during postnatal development (Lehmenkühler et al., 1993; Prokopová et al., 1997; Voříšek and Syková, 1997a,b). The ECS volume in the cortex is about twice as large ($\alpha = 0.36$–0.46) in the newborn rat as in the adult rat ($\alpha = 0.21$–0.23) (Fig. 4A and B). The reduction in the ECS volume fraction correlates well with the growth of blood vessels. The larger ECS in the first days of postnatal development can be attributed to incomplete neuronal migration, gliogenesis and angiogenesis and to the presence of large extracellular matrix proteoglycans, particularly hyaluronic acid, which due to the mutual repulsion of its highly negatively charged branches occupies a great deal of space and holds cells apart. In rat spinal cord gray matter, α decreases with neuronal development and gliogenesis from postnatal day 4–12 by about 15%, while λ significantly increases, showing that the diffusion of molecules becomes more hindered with age. The large ECS channels during development may allow the migration of larger substances (e.g. growth factors) and provide better conditions for cell migration during development. On the other hand, the large ECS in the neonatal brain could significantly dilute ions, metabolites and neuroactive substances released from cells, relative to release in adults, and may be a factor in the prevention of anoxic injury, seizure and spreading depression in young individuals. The diffusion parameters could also play an important role in the developmental process itself. Diffusion parameters are substantially different in myelinated and unmyelinated white matter (Prokopová et al., 1997; Voříšek and Syková, 1997a). In myelinated spinal cord white matter or in corpus callosum, the tortuosity is higher when TMA^+ diffuses across the axons than when it diffuses along the fibers (Fig. 2C). Isotropic diffusion is found in the corpus callosum and spinal cord white matter of young rats with incomplete myelination.

Similar changes in diffusion anisotropy and in ECS volume have been found during demyelinating diseases. Human inflammatory demyelinating diseases, e.g. multiple sclerosis, are characterized by a loss of diffusion anisotropy. Experimental autoimmune encephalomyelitis (EAE) is widely used as an animal model for multiple sclerosis (for review, see Lassmann, 1983; Lassmann et al., 1986). In experiments in which EAE was induced by an injection of guinea-pig myelin basic protein, typical morphological changes in the CNS tissue could be observed including demyelination, an inflammatory reaction, astrogliosis, blood–brain barrier damage as well as paralysis at 14–17 days post-injection (Šimonová et al., 1996). The TMA^+ method revealed that EAE was accompanied by increases in ECS volume fraction in all regions of the spinal cord (Fig. 4E), namely in the dorsal horn (from 0.21 to 0.28), ventral horn (from 0.23 to 0.47), the intermediate region (from 0.22 to 0.33) and in white matter (from 0.18 to 0.30). The enlarged ECS volume was accompanied by a decrease in tortuosity and a decrease in non-specific uptake. There was a close correlation between the changes in ECS diffusion parameters and the manifestation of neurological signs (paraparesis, paraplegia), which were preceded and greatly outlasted by the astrogliosis and inflammatory reaction (Šimonová et al., 1996). These results indicate that an increase in ECS volume may also alter the diffusion parameters of nervous tissue during inflammatory and demyelinating diseases, and may thus decrease the efficacy of synaptic as well as non-synaptic transmission and intercellular communication during these disorders.

1.5. Diffusion parameters and the extracellular matrix

The solution in the ECS is not a simple salt solution. It has become apparent that long chain polyelectrolytes, either attached or unattached to cellular membranes, are present in the ECS. The ECS contains a number of glycosaminoglycans (e.g. hyaluronate), glycoproteins and proteoglycans that constitute the extracellular matrix (ECM). Various ECM molecules and adhesion molecules have also been described, e.g. fibronectin, tenascin, laminin, etc. (Thomas and Steindler, 1995; Celio et al., 1998), the content of which can dynamically change during development, aging, wound healing and many pathological processes. ECM molecules are produced by both neurons and glia. These molecules have been suggested to cordon off distinct functional units in the CNS (groups of neurons, axon tracts, and nuclear groups). As shown in Fig. 1, these large molecules can slow down the movement (diffusion) of various neuroactive substances through the ECS. Recently, we have shown that tenascin-R- or tenascin-C-deficient mice have not only a significantly lower tortuosity, but also smaller ECS volume fraction

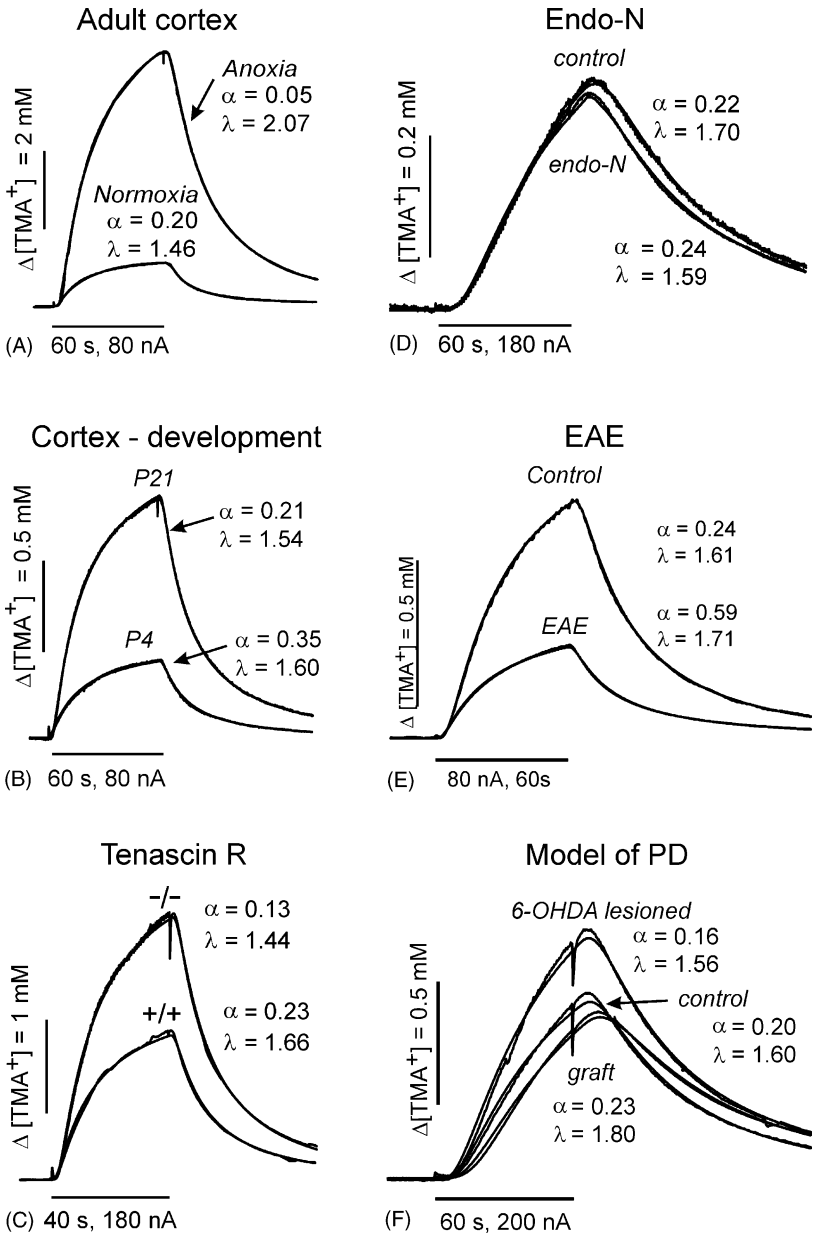

Fig. 4. (A) TMA^+ diffusion curves and the corresponding values of volume fraction (α) and tortuosity (λ) obtained in the rat cortex in vivo during normoxia and during anoxia, (B) in the rat cortex during development on the 4th and 21st postnatal days and (C) in the cortex of Tenascin-R knockout (−/−) and control (+/+) mice. (D) The effect of endo-neuraminidase injection on the diffusion curve recorded in the cortex of an adult (3 month-old) rat. Clostridium neuraminidase (5 μU) dissolved in 500 nl of ACF was applied for 10 min by pressure injection using a micropipette glued to the measuring electrode array at an inter-tip distance of 500 μm. Note that after neuraminidase application, tortuosity decreased. (E and F) Typical TMA^+ diffusion curves and corresponding values of α and λ. (E) Typical recordings from the spinal ventral horn in a control rat and in a rat with experimental autoimmune encephalomyelitis (EAE). Note the high α and λ in the EAE animal. (F) Typical diffusion curves in the striatum of a control rat, in a 6-OHDA-lesioned and in a grafted animal. Note that both α and λ were lower in the striatum of the 6-OHDA-lesioned rat than in the control animal and higher inside the graft compared with the areas of the 6-OHDA-lesioned striatum.

(Antonova et al., 2001) (Fig. 4C). Diffusion-weighted NMR also revealed a decrease in ADC_W in Tenascin-deficient mice (Syková and Voříšek, unpublished data). This suggests that these molecules are important for keeping cellular structures apart, i.e. maintaining the ECS at its optimal size. The size of the ECS volume plays an important role in volume transmission and might play an important role in the synaptic spillover of glutamate and GABA. Indeed, it has been shown that tenascin-R knockout mice lack perineuronal nets, which leads to a decrease in the activity of parvalbumin-containing interneurons and a reduction in the perisomatic inhibition of hippocampal pyramidal cells (Saghatelyan et al., 2001), resulting in increased basal excitatory synaptic transmission and reduced long-term potentiation (LTP) (Bukalo et al.,

2001). Tenascin-C deficient mice also show reduced LTP after theta-burst stimulation and no long-term depression (LTD) after low-frequency stimulation (Evers et al., 2002). Importantly, these molecules can hinder the diffusion of molecules so that they are confined to certain places, while diffusion to other brain regions will be facilitated.

Modification of the extracellular matrix can also be achieved by enzymatic treatment. Hyaluronic acid and chondroitin sulphate proteoglycans are essential components of the extracellular matrix, forming so-called perineuronal nets surrounding neurons in the cortex and hippocampus. There is also increasing evidence that N-CAM, the protein backbone of polysialic acid (PSA), is involved in synaptic plasticity. PSA, which is almost exclusively carried by N-CAM, is a major modulator of cell adhesion and is highly expressed in areas of continuous neurogenesis, neuronal migration, neurite extension and synapse formation. It has been found that mice treated with chondroitinase ABC or with antibodies against N-CAM and transgenic mice lacking the N-CAM gene have impaired LTP (Becker et al., 1996; Muller et al., 1996). It has also been demonstrated that hydrated PSA influences a sufficiently large volume at the cell surface to exert broad steric effects, and that the removal of PSA causes a detectable change in the intercellular space. By contrast, chondroitin sulphate has been found to have little influence on the intercellular space (Yang et al., 1992). We therefore used a single intracortical injection of endoneuroaminidase NE (endo-N, 10 U/I, 5 μU) or chondroitinase ABC (10 μU) and studied the acute (3 h) and chronic (24 h) effects of this treatment on ECS diffusion parameters. Fig. 4D shows the effect of endo-N in rat cortex. A significant decrease in tortuosity is already found in the ipsilateral hemisphere 2 h after injection and persists at 24 h (Syková, 2001a). There is also a small but significant increase in the ECS volume fraction in the first 3 h after injection that is not found at 24 h. On the other hand, incubation of isolated rat spinal cord with 0.1% hyaluronic acid increases tortuosity in the spinal dorsal horns (Syková, 2003).

1.6. Activity-related changes in ECS volume and geometry

Transmembrane ionic fluxes during neuronal activity are accompanied by the movement of water and by cellular swelling. This results in changes in ECS diffusion parameters, ECS volume decrease, tortuosity increase and ADC decrease. In the spinal cord of the rat or frog, repetitive electrical stimulation results in an ECS volume decrease from about 0.24–(0.12–0.17), i.e. the ECS volume decreases by as much as 30–50% (Syková, 1987; Svoboda and Syková, 1991). The ECS volume in the spinal dorsal horn of the rat also decreases by 20–50% after injury of the ipsilateral hind paw evoked by subcutaneous injection of turpentine or after thermal injury (Svoboda and Syková, 1991). The changes in ECS diffusion parameters can persist for many minutes (30 min after electrical stimulation or even 120 min after peripheral injury) after stimulation has ceased, suggesting long-term changes in neuronal excitability, neuron-glia communication and volume transmission.

1.7. Lactation

In the supraoptic nucleus a significant reduction in the astrocytic coverage of hypothalamic magnocellular neurons occurs under specific physiological conditions such as lactation (Theodosis and Poulain, 1993; Hatton, 1997). We tested whether such a change modifies the extracellular space diffusion parameters in acute slices from virgin and lactating rats. Measurements were performed along three perpendicular axes (x, y and z) in the supraoptic nucleus using the real-time TMA^+ ionotophoretic method. The values of both λ and α were reduced in lactating animals due to the retraction of astrocytic processes and this resulted in enhanced diffusion (Vargová et al., 2002). These findings indicate that the supraoptic nucleus is an anisotropic structure in which the diffusion parameters are modified during lactation. Whereas the reduction in λ in lactating animals may reflect the relative absence of astrocytic processes around neurons, the diminished α could result from the retraction of astrocytic processes and the enhanced proportion of direct neuronal membrane juxtapositions. The plastic changes occurring in the supraoptic nucleus during lactation facilitate the diffusion of neuroactive substances and may increase cross-talk between synapses. Under these conditions, glutamate spillover, monitored through the metabotropic glutamate receptor (mGluR)-mediated depression of GABAergic transmission, was greatly enhanced. Conversely, impeding diffusion with dextran largely prevented crosstalk between glutamatergic and GABAergic afferent inputs (Vargová et al., 2002, 2003). Astrocytes, therefore, by hindering diffusion in the extracellular space, regulate intersynaptic communication between neighboring synapses, and, probably, overall volume transmission in the brain.

1.8. ECS diffusion parameters during anoxia/ischemia

Dramatic K^+ and pH_e changes and cell swelling occur in the brain and spinal cord during anoxia and/or ischemia (Syková, 1992; Syková et al., 1994; Xie et al., 1995). Within 2 min after respiratory arrest in adult rats, blood pressure starts to decrease, the pH_e decreases to pH 6.4–6.6 and $[K^+]_e$ increses to about 50–70 mM. A negative DC slow potential shift is accompanied by a decrease in ECS volume fraction to 0.04–0.07 (Syková et al., 1994).

Fig. 4A shows that during hypoxia and terminal anoxia, the ECS volume fraction in rat cortex or spinal cord decreases from about 0.20 to about 0.05, while tortuosity increases from 1.5 to about 2.1 (Syková et al., 1994; Lundbaek and Hansen, 1992). The same ultimate changes were found in all neonatal, adult and aged rats, in grey and white matter, in the cortex, corpus callosum and spinal cord. However, the time course in white matter was significantly slower than in

gray matter; and the time course in neonatal rats was about 10 times slower than in adults (Vořišek and Syková, 1997b). Linear regression analysis revealed a positive correlation between the normoxic size of the ECS volume and the time course of the changes. This corresponds to the well-known resistance of the immature CNS and the greater susceptibility of the aged brain to anoxia.

Acute insults, such as fast terminal anoxia or the application of 50 mM K^+ to the brain slice, evoked a decrease in both α and the ADC of TMA^+ (increase in λ), and the time courses strongly correlated with the time course of a decrease in the ADC_W as determined by DW-MRI (Van der Toorn et al., 1996). This is consistent with measurements during chronic changes related to neuronal death and astrogliosis, where α increased while ADC_{TMA} and ADC_W decreased (λ increased), but the time courses of these changes were different.

Recently, TMA measurements revealed transient changes in ECS diffusion parameters after recovery from transient ischemia (Zoremba et al., 2003). The volume fraction in postischemic cortex increased to about 0.30, while λ was unchanged. This increase was apparently due to the development of postischemic cytotoxic oedema.

1.9. Diffusion parameters during astrogliosis and in injured brain

Ions as well as neurotransmitters released into the ECS during neuronal activity or pathological states interact not only with postsynaptic and presynaptic membranes, but also with extrasynaptic receptors, including those on glial cells. Stimulation of glial cells leads to the activation of ion channels, second messengers and intracellular metabolic pathways, and to changes in glial volume that are accompanied by dynamic variations in ECS volume, particularly the swelling and possible re-arrangement of glial processes. In addition to their role in the maintenance of extracellular ionic homeostasis, glial cells may thus, by regulating their volume, influence the extracellular pathways for neuroactive substances.

Glial swelling resulting in an ECS volume fraction decrease contributes to the mechanism of nonspecific feedback suppressing neuronal excitability (Syková, 1997) (Fig. 5). Active neurons release K^+, which accumulates in the ECS, depolarizes glial cells and is taken up by glia. In depolarized glial cells an alkaline shift is evoked, e.g. by the activation of Na^+/HCO_3^- cotransport. This alkaline shift in glial pH_i causes an acid shift in pH_e. Extracellular acidosis further suppresses neuronal activity. Transmembrane ionic movements are accompanied by water and therefore result in glial swelling (greater and faster in the adult brain than during development). A decrease in ECS volume leads to a greater accumulation of ions and neuroactive substances, the “crowding” of molecules of the extracellular matrix in the ECS and decreases in the ADCs of various neuroactive molecules.

Animal models that have been developed to study morphological changes during brain injury include a cortical stab wound. This model of cortical injury, similar to other models, elicits reactive gliosis involving both the hyperplasia and hypertrophy of astrocytes, which show intense staining for glial fibrillary acidic protein (GFAP). Astrogliosis is a characteristic response to cortical stab wounds in rodents (Norton et al., 1992). We studied the diffusion parameters in rat cortex 3–35 days following a cortical stab wound, using diffusion-weighted MR to determine the apparent diffusion coefficient of water (ADC_W) in the tissue and the real-time TMA^+ method to measure the extracellular space (ECS) diffusion parameters: ECS volume fraction α and the apparent diffusion coefficient of TMA^+ (ADC_{TMA}). Severe astrogliosis was found close to the wound, mild astrogliosis in the ipsilateral but not the contralateral cortex (Fig. 3: GFAP). Chondroitin sulfate proteoglycan (CSPG) expression was increased throughout the ipsilateral cortex (Fig. 3: CSPG). In the hemisphere contralateral to the wound, α, ADC_{TMA} and ADC_W were not significantly different from control values. ECS volume fraction was increased only in the vicinity of the wound, in the region of cell death and severe astrogliosis, at 3 and 7 days after injury. Wounding was typically accompanied by an ECS volume increase and a substantial tortuosity increase to mean values of α of about 0.26 and λ of about 1.77 (Roitbak and Syková, 1999).

However, both ADC_{TMA} and ADC_W were significantly decreased after lesioning in the vicinity of the wound as well as in the rest of the ipsilateral hemisphere distant from the wound (Fig. 3: ADC_W map). Thus, both ADC_W and ADC_{TMA} decreased in regions with no change in α, but where CSPG expression increased. An increase in extracellular matrix expression may therefore impose diffusion barriers not only for TMA^+ molecules but also for water.

1.10. Diffusion parameters in grafted tissue

Similarly, both the size of the ECS (α) and, surprisingly, also λ were significantly higher in cortical grafts than in host cortex, about 0.35 and 1.79, respectively, as was also the case in gliotic cortex after a stab wound. Both α and λ were increased in cortical grafts of fetal tissue transplanted to the midbrain, where severe astrogliosis compared to host cortex was found, but not in fetal grafts placed into a cavity in the cortex, where only mild astrogliosis occurred (Syková et al., 1999). Another characteristic feature of cortical grafts into the midbrain was the variability of α and λ. The different values found at various depths of the grafts correlated with the morphological heterogeneity of the graft neuropil. These measurements showed that even when the ECS in gliotic tissue or in cortical grafts is larger than in normal cortex, the tortuosity is still higher, and the diffusion of chemical signals in such tissue may be hindered. Limited diffusion may thus have a negative impact on the viability of grafts in host brains. Compared to host cortex, immunohistochemistry showed myelinated patches

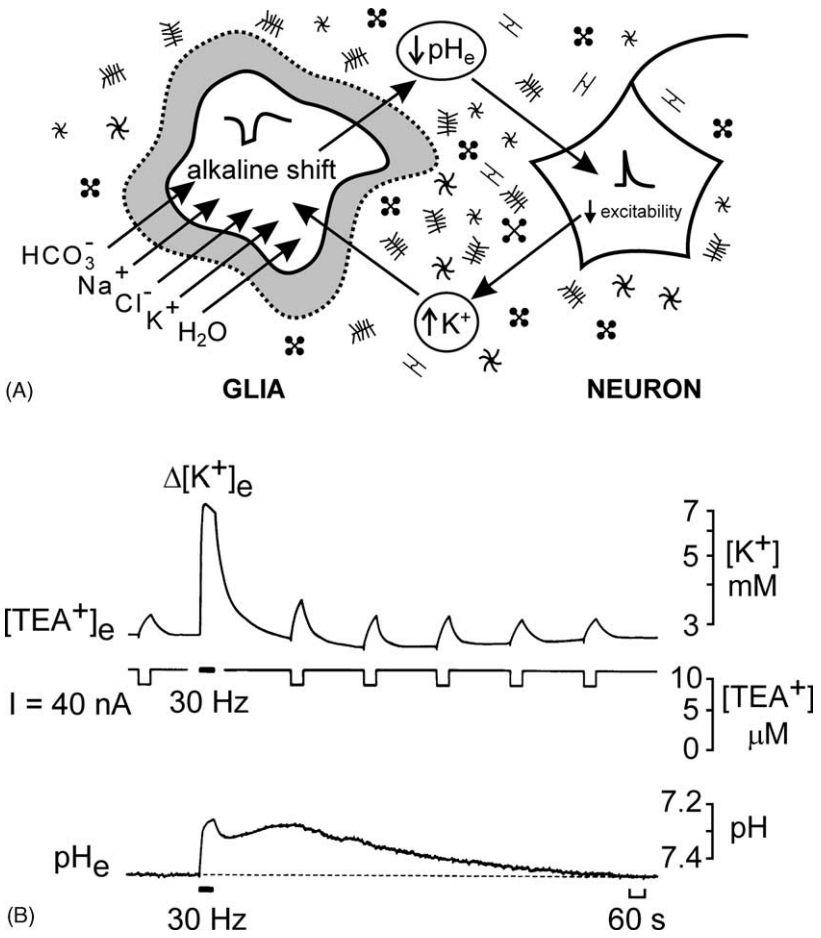

Fig. 5. (A) Schematic of the mechanism of nonspecific feedback suppressing neuronal excitability. Active neurons release K^+, which accumulates in the ECS and depolarizes glial cells. This causes an alkaline shift in glial pH_i and an acid shift in pH_e. Extracellular acidosis further suppresses neuronal activity. Transmembrane ionic movements accomapnied by water, result in glial swelling, ECS volume decrease and in the ever-increasing accumulation of ions and neuroactive substances in the ECS untill the neuronal activity stops. B: Effect of repetitive stimulation of afferent input on TEA^+ diffusion curves, extracellular K^+ and pH in isolated frog spinal cord. Higher diffusion curves indicate an ECS volume decrease due to cell swelling outlasting the 1 min repetitive stimulation (30 Hz) of the dorsal root for 30 min. Note the extracellular K^+ increase and the acid shift evoked by stimulation. The time course of the acid shift correlates with the decrease in ECS volume.

and a larger number of hypertrophic astrocytes in areas of high λ values, suggesting that more numerous and/or thicker glial cell processes might be a cause of the increased tortuosity.

Functional recovery after the transplantation of dopaminergic cells into the lesioned striatum is dependent on the widespread diffusion of the transmitter released by the graft. We investigated the diffusion parameters of the extracellular space in the striatum of 6-hydroxydopamine-lesioned and intrastriatally grafted rats in vivo. Compared with controls, both α and λ were lower in lesioned animals ($\alpha = 0.14$, $\lambda = 1.50$). In both macro- and micro-grafts, α returned to normal control values ($\alpha = 0.24$); however, λ increased ($\lambda = 1.80$) (Fig. 4F; Reum et al., 2002). Graft deposits, in contrast to their enhanced capacity for dopaminergic reinnervation, impair the conditions for diffusion and extrasynaptic transmission in the striatum, presumably because of extensive tissue damage, cell loss, and astrogliosis.

1.11. ECS diffusion parameters during aging

Aging in nervous tissue, particularly in the hippocampus and cortex, is accompanied by various degenerative processes including a decreased number and efficacy of synapses, a decrease in transmitter release, neuronal loss, astrogliosis, demyelination, deposits of β amyloid, changes in extracellular matrix proteins, etc. These and other changes affect the ECS diffusion parameters. Using the TMA^+ method, the ECS diffusion parameters α, $\lambda_{x,y,z}$ and k' were measured in the cortex, corpus callosum and hippocampus (CA1, CA3 and in dentate gyrus) of aged rats. If diffusion in a particular brain region is anisotropic, then the correct value of the ECS volume fraction cannot be calculated from measurements done only in one direction (Rice et al., 1993; Nicholson and Syková, 1998; Syková et al., 2002). We performed the measurements in three privileged orthogonal directions (*x*, transversal; *y*, sagital; *z*, vertical).

In all three regions—cortex, corpus callosum and hippocampus—the mean ECS volume fraction α was significantly lower in aged rats (26–32 months old), ranging from 0.16 to 0.18, than in young adults (3–4 months old) in which α ranged from 0.22 to 0.24 (Fig. 6). Nonspecific uptake k' was also significantly lower in aged rats. From Fig. 6A and B it is evident that the diffusion curves for the hippocampus are larger in the aged rat than in the young one, i.e. the space available for TMA^+ diffusion is smaller. Although the mean λ values along the *x*-axis were not significantly different between young and aged rats, the values were significantly lower in aged rats along the *y*- and *z*-axes (Fig. 6A and B). This shows that there is a loss of anisotropy in the aging hippocampus, particularly in the CA3 region and the dentate gyrus (Syková et al., 1998).

The three-dimensional pattern of diffusion away from a point source can be illustrated by constructing iso-concentration spheres (isotropic diffusion) or ellipsoids

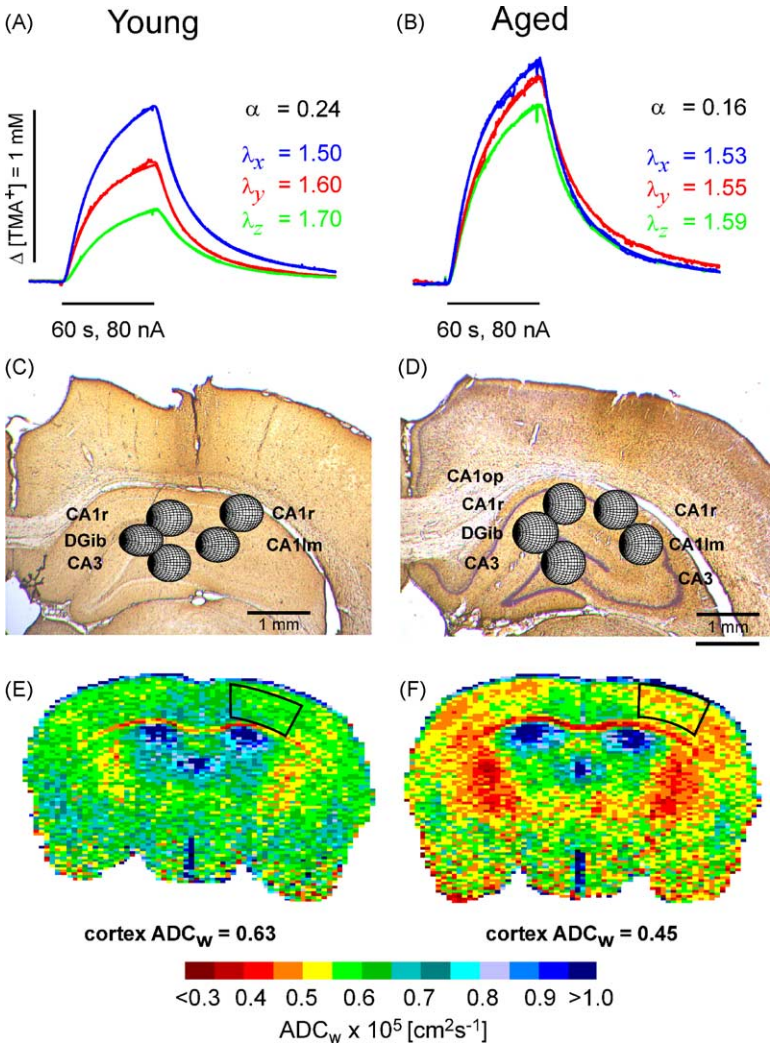

Fig. 6. Diffusion parameters in the dentate gyrus of the hippocampus differ between a young adult and an aged rat. (A) Anisotropic diffusion in the dentate gyrus of a young adult rat. TMA^+ diffusion curves were measured along three orthogonal axes (*x*, mediolateral; *y*, rostrocaudal; *z*, dorsoventral). The slower rise in the *z*-axis than in the *y*-axis and in the *y*-axis than in the *x*-axis indicates a higher tortuosity and more restricted diffusion. The amplitude of the curves shows that the TMA^+ concentration, at approximately the same distance from the tip of the iontophoresis electrode, is much higher along the *x*-axis than along the *y*-axis and even higher than along the *z*-axis (λ_x, λ_y, λ_z). Note that the actual ECS volume fraction α is about 0.2 and can be calculated only when measurements are done along the *x*-, *y*- and *z*-axes. (B) Anisotropy is almost lost in an aged rat; the diffusion curves are higher, showing that α is smaller. (C and D) Iso-concentration surfaces for a 0.1 mM TMA^+ concentration contour 60 s after the onset of a 80 nA iontophoretic pulse in different regions within the hippocampus stained for GFAP. The surfaces were generated using mean values of volume fraction and tortuosity. (C) The ellipsoids represent anisotropic diffusion in the dentate gyrus of a young adult rat. (D) The larger spheres in an aged rat, corresponding to isotropic diffusion and to a lower ECS volume fraction, demonstrate that diffusion from any given source will lead to a higher concentration of substances in the surrounding tissue and a larger action radius in aged rats than in young adults. (E) Pseudocolor image showing a typical ADC_W map of a young (8-month old) mouse. (F) Pseudocolor image showing a typical ADC_W map of an aged (21-month old) mouse. The scale below the ADC_W maps shows the relation between the intervals of ADC_W values and the colors used for visualization. Note the decrease of ADC_W in most of the brain areas of an aged mouse, apparently related to a decrease in the ECS volume.

(anisotropic diffusion) for extracellular TMA^+ concentration. The surfaces in Fig. 6C and D represent the locations where the TMA^+ concentration first reached 1 mM, 60 s after its application in the center. The ellipsoid in the hippocampus of the young adult rat reflects the different abilities of substances to diffuse along the *x*-, *y*- and *z*-axes, while the sphere in the hippocampus of the aged rat shows isotropic diffusion.

Morphological changes during aging include cell loss, loss of dendritic processes, demyelination, astrogliosis, swollen astrocytic processes and changes in the extracellular matrix. Indeed, we found that there is a significant decrease in the ADC of many neuroactive substances, including ADC_W, in the aging brain, which accompanies astrogliosis and changes in the extracellular matrix (Fig. 6C and D). One of the explanations why α in the cortex, corpus callosum and hippocampus of senescent mice and rats is significantly lower than in young adults could be astrogliosis in the aged brain. Increased GFAP staining and an increase in the size and fibrous character of astrocytes have been found in the cortex, corpus callosum and hippocampus of senescent rats, which may account for changes in the ECS volume fraction (Syková et al., 1998). Other changes could account for the decreases in λ values and for the loss of tissue anisotropy. We found decreased staining for chondroitin sulfate proteoglycans and for fibronectin, i.e. a loss of extracellular matrix macromolecules. If compared with tenascin knockout mice, the data are similar. In addition we found in the hippocampus in the CA1, CA3, as well as in the dentate gyrus changes in the arrangement of fine astrocytic processes. These are normally organized in parallel in the *x*–*y* plane, but this organization totally disappeared during aging (Syková et al., 1998, 2002).

Volume fraction is thus decreasing during the entire postnatal life. The smaller ECS during aging can also affect cell migration (tumor cells as well as stem cells). The ensuing decrease in the ECS size could be explained by the disappearance of a significant part of the ECS matrix, reduced dendritic trees, demyelination and the proliferation of glia, neuronal degeneration, the loss of extracellular matrix and astrogliosis.

The observed loss of anisotropy in senescent rats could therefore not only lead to impaired cortical and, particularly, hippocampal function. The decrease in ECS size could be responsible for the greater susceptibility of the aged brain to pathological events (particularly ischemia) (Syková et al., 2002), the poorer outcome of clinical therapy and the more limited recovery of affected tissue after insult.

The hippocampus is well known for its role in memory formation, especially declarative memory. Our studies revealed that the degree of learning deficit in aged rats correlates with the changes in α, λ and k' (Syková et al., 2002). It is therefore reasonable to assume that diffusion anisotropy, which leads to a certain degree of specificity in extrasynaptic communication, may play an important role in memory formation. There was a significant difference between mildly and severely behaviorally impaired rats (rats were tested in a Morris water maze). The ECS in the dentate gyrus of severely impaired rats was significantly smaller and anisotropy was more reduced than in mildly impaired rats. Anisotropy might be important for extrasynaptic transmission by channeling the flux of substances in a preferential direction. Its loss may severely disrupt extrasynaptic communication in the CNS, which has been suggested to play an important role in memory formation (Nicholson and Syková, 1998; Syková, 2001b, 2002; Syková et al., 1998).

2. Conclusion

The movement of neuroactive substances in the CNS is hindered by the cell swelling-induced narrowing of intercellular clefts and by diffusion barriers formed by molecules of the ECS matrix, by the swelling of fine glial processes and by astrogliosis. It is also evident that extracellular water diffusion is hindered not only by the size of the pores between the cells, but also by the cellular structure, including the fine swelling and movement of glial processes towards active synapses and changes in extracellular space diffusion barriers—extracellular matrix molecules (quadrupartite synapse). The extracellular matrix, besides its apparent importance for tissue anisotropy, is also important for maintaining a relatively large ECS volume. The changes in ECS diffusion parameters in physiological and pathological states can affect the efficacy of synaptic as well as extrasynaptic transmission, induce damage to nerve cells by the increased accumulation of toxic substances, as well as be an important factor to consider when using diffusion-weighted NMR for diagnostic purposes or when considering therapeutic drug application.

References

Agnati, L.F., Zoli, M., Stromberg, I., Fuxe, K., 1995. Intercellular communication in the brain: wiring versus volume transmission. Neuroscience 69, 711–726.

Antonova, T., Mazel, T., Syková, E., Vargová, L., 2001. Extracellular space volume and diffusion barriers in the CNS: the role of extracellular matrix, cell swelling and astrogliosis. In: Proceedings of the 34th International Congress of Physiological Societies (abstracts of papers). Christchurch.

Asztely, F., Erdemli, G., Kullmann, D.M., 1997. Extrasynaptic glutamate spillover in the hippocampus: dependence on temperature and the role of active glutamate uptake. Neuron 18, 281–293.

Becker, C.G., Artola, A., Gerardy-Schahn, R., Becker, T., Welzl, H., Schachner, M., 1996. The polysialic acid modification of the neural cell adhesion molecule is involved in spatial learning and hippocampal long-term potentiation. J. Neurosci. Res. 45, 143–152.

Benveniste, H., Hedlund, L.W., Johnson, G.A., 1992. Mechanism of detection of acute cerebral ischemia in rats by diffusion-weighted magnetic resonance microscopy. Stroke 23, 746–754.

Bukalo, O., Schachner, M., Dityatev, A., 2001. Modification of extracellular matrix by enzymatic removal of chondroitin sulfate and by lack of tenascin-R differentially affects several forms of synaptic plasticity in the hippocampus. Neuroscience 104, 359–369.

Celio, M.R., Spreafico, R., De Biasi, S., Vitellaro-Zuccarello, L., 1998. Perineuronal nets: past and present. Trends Neurosci. 21, 510–515.

Chvátal, A., Berger, T., Voříšek, I., Orkand, R.K., Kettenmann, H., Syková, E., 1997. Changes in glial K^+ currents with decreased extracellular volume in developing rat white matter. J. Neurosci. Res. 49, 98–106.

Chvátal, A., Andìrová, M., Žiak, D., Syková, E., 1999. Glial depolarization evokes a larger potassium accumulation around oligodendrocytes than around astrocytes in gray matter of rat spinal cord slices. J. Neurosci. Res. 56, 493–505.

Evers, M.R., Salmen, B., Bukalo, O., Rollenhagen, A., Bosl, M.R., Morellini, F., Bartsch, U., Dityatev, A., Schachner, M., 2002. Impairment of L-type Ca2+ channel-dependent forms of hippocampal synaptic plasticity in mice deficient in the extracellular matrix glycoprotein tenascin-C. J. Neurosci. 22, 7177–7194.

Fuxe, K., Agnati, L.F., 1991. Volume Transmission in the Brain: Novel Mechanisms for Neural Transmission. Raven Press, New York.

Hatton, G.I., 1997. Function-related plasticity in hypothalamus. Ann. Rev. Neurosci. 20, 375–397.

Jarvis, C., Lilge, L., Vipond, G.J., Andrew, R.D., 1999. Interpretation of intrinsic optical signals and calcein fluorescence during acute excitotoxic insult in the hippocampal slice. NeuroImage 10, 357–372.

Korf, J., Klein, H.C., Postrema, F., 1988. Increases in striatal and hippocampal impedance and extracellular levels of amino acids by cardiac arrest in freely moving rats. J. Neurochem. 50, 1087–1096.

Kullmann, D.M., Erdemli, G., Asztely, F., 1996. LTP of AMPA and NMDA receptor-mediated signals: evidence for presynaptic expression and extrasynaptic glutamate spill-over. Neuron 17, 461–474.

Lassmann, H., 1983. Comparative Neuropathology of Chronic Experimental Allergic Encaphalomyelitis and Multiple Sclerosis. Springer, Berlin.

Lassmann, H., Vass, K., Brunner, C., Seitelberger, F., 1986. Characterization of inflammatory infiltrates in experimental allergic encephalomyelitis. Prog. Neuropathol. 6, 33–62.

Latour, L.L., Svoboda, K., Mitra, P.P., Sotak, C.H., 1994. Time-dependent diffusion of water in a biological model system. Proc. Natl. Acad. Sci. USA 91, 1229–1233.

Lehmenkühler, A., Syková, E., Svoboda, J., Zilles, K., Nicholson, C., 1993. Extracellular space parameters in the rat neocortex and subcortical white matter during postnatal development determined by diffusion analysis. Neuroscience 55, 339–351.

Lundbaek, J.A., Hansen, A.J., 1992. Brain interstitial volume fraction and tortuosity in anoxia. Evaluation of the ion-selective microelectrode method. Acta Physiol. Scand. 146, 473–484.

Matsuoka, Y., Hossmann, K.A., 1982. Cortical impedance and extracellular volume changes following middle cerebral artery occlusion in cats. J. Cerebral Blood Flow Metabol. 2, 466–474.

Mazel, T., Šimonová, Z., Syková, E., 1998. Diffusion heterogeneity and anisotropy in rat hippocampus. NeuroReport 9, 1299–1304.

Muller, D., Wang, C., Skibo, G., Toni, N., Cremer, H., Calaora, V., Rougon, G., Kiss, J.Z., 1996. PSA-NCAM is required for activity-induced synaptic plasticity. Neuron 17, 413–422.

Nicholson, C., 2001. Diffusion and related transport mechanisms in brain tissue. Rep. Prog. Phys. 64, 815–884.

Nicholson, C., Phillips, J.M., 1981. Ion diffusion modified by tortuosity and volume fraction in the extracellular microenvironment of the rat cerebellum. J. Physiol. (London) 321, 225–257.

Nicholson, C., Tao, L., 1993. Hindered diffusion of high molecular weight compounds in brain extracellular microenvironment measured with integrative optical imaging. Biophys. J. 65, 2277–2290.

Nicholson, C., Syková, E., 1998. Extracellular space structure revealed by diffusion analysis. Trends NeuroScience 21, 207–215.

Norris, D.G., Niendorf, T., Leibfritz, D., 1994. Healthy and infarcted brain tissues studied at short diffusion times: the origins of apparent restriction and the reduction in apparent diffusion coefficient. NMR Biomed. 7, 304–310.

Norton, W.T., Aquino, D.A., Hosumi, I., Chiu, F.C., Brosnan, C.F., 1992. Quantitative aspects of reactive gliosis: a review. Neurochem. Res. 17, 877–885.

Prokopová, Š., Vargová, L., Syková, E., 1997. Heterogeneous and anisotropic diffusion in the developing rat spinal cord. NeuroReport 8, 3527–3532.

Prokopová-Kubinová, Š., Vargová, L., Tao, L., Ulbrich, K., Šubr, V., Syková, E., Nicholson, C., 2001. Poly[N-(2-hydroxypropyl)methacrylamide] polymers diffuse in brain extracellular space with same tortuosity as small molecules. Biophys. J. 80, 542–548.

Reum, T., Olshausen, F., Mazel, T., Voříšek, I., Morgenstern, R., Syková, E., 2002. Diffusion parameters in the striatum of rats with 6-hydroxydopamine-induced lesions and with fetal mesencephalic grafts. J. Neurosci. Res. 70, 680–693.

Rice, M.E., Okada, Y.C., Nicholson, C., 1993. Anisotropic and heterogeneous diffusionin the turtle cerebellum: implications for volume transmission. J. Neurophysiol. 70, 2035–2044.

Roitbak, T., Syková, E., 1999. Diffusion barriers evoked in the rat cortex by reactive astrogliosis. Glia 28, 40–48.

Saghatelyan, A.K., Dityatev, A., Schmidt, S., Schuster, T., Bartsch, U., Schachner, M., 2001. Reduced perisomatic inhibition, increased excitatory transmission, and impaired long-term potentiation in mice deficient for the extracellular matrix glycoprotein tenascin-R. Mol. Cell. Neurosci. 17, 226–240.

Šimonová, Z., Svoboda, J., Orkand, P., Bernard, C.C.A., Lassmann, H., Syková, E., 1996. Changes of extracellular space volume and tortuosity in the spinal cord of Lewis rats with experimental autoimmune encephalomyelitis. Physiol. Res. 45, 11–22.

Svoboda, J., Syková, E., 1991. Extracellular space volume changes in the rat spinal cord produced by nerve stimulation and peripheral injury. Brain Res. 560, 216–224.

Syková, E., 1987. Modulation of spinal cord transmission by changes in extracellular K^+ activity and extracellular volume. Can. J. Physiol. Pharmacol. 65, 1058–1066.

Syková, E., 1992. Ionic and Volume Changes in the Microenvironment of Nerve and Receptor Cells. In: Ottoson, D. (Ed.), Progress in Sensory Physiology. Springer-Verlag, Heidelberg, pp. 1–167.

Syková, E., 1997. The extracellular space in the CNS: its regulation, volume and geometry in normal and pathological neuronal function. Neuroscientist 3, 28–41.

Syková, E., 2001a. Glial diffusion barriers during aging and pathological states. Prog. Brain Res. 132, 339–363.

Syková, E., 2001b. Glia and extracellular space diffusion parameters in the injured and aging brain. In: de Vellis, J. (Ed.), Neuroglia in the Aging Brain. Humana Press, Totowa, pp. 77–98.

Syková, E., 2002. Plasticity of the extracellular space. In: Walz, W. (Ed.), The Neuronal Microenvironment. Humana Press, Totowa, pp. 57–81.

Syková, E., 2003. Diffusion Parameters of the Extracellular Space. Israel J. Chem., 43.

Syková, E., Chvátal, A., 2000. Glial cells and volume transmission in the CNS. Neurochem. Int. 36, 397–409.

Syková, E., Svoboda, J., Polák, J., Chvátal, A., 1994. Extracellular volume fraction and diffusion characteristics during progressive ischemia and terminal anoxia in the spinal cord of the rat. J. Cerebral Blood Flow Metabol. 14, 301–311.

Syková, E., Mazel, T., Šimonová, Z., 1998. Diffusion constraints and neuron-glia interaction during aging. Exp. Gerontol. 33, 837–851.

Syková, E., Mazel, T., Roitbak, T., Šimonová, Z., 1999. Morphological changes and diffusion barriers in auditory cortex and hippocampus of aged rats. In: Proceedings of the 22nd Midwinter Research Meeting of the Association for Research in Otolaryngology (abstracts of papers). St. Petersburg Beach.

Syková, E., Roitbak, T., Mazel, T., Šimonová, Z., Harvey, A.R., 1999. Astrocytes, oligodendroglia, extracellular space volume and geometry in rat fetal brain grafts. Neuroscience 91, 783–798.

Syková, E., Mazel, T., Vargová, L., Voříšek, I., Prokopová, Š., 2000. Extracellular space diffusion and pathological states. Prog. Brain Res. 125, 155–178.

Syková, E., Mazel, T., Hasenöhrl, R.U., Harvey, A.R., Šimonová, Z., Mulders, W.H.A.M., Huston, J.P., 2002. Learning deficits in aged rats related to decrease in extracellular volume and loss of difussion anisotropy in hippocampus. Hippocampus 12, 469–479.

Syková, E., Vargová, L., Kubinová, Š., Jendelová, P., Chvátal, A., 2003. The relationship between changes in intrinsic optical signals and cell swelling in rat spinal cord slices. NeuroImage 18, 214–230.

Tao, L., Masri, D., Hrabetova, S., Nicholson, C., 2002. Light scattering in rat neocortical slices differs during spreading depression and ischemia. Brain Res. 952, 290–300.

Theodosis, D.T., Poulain, D.A., 1993. Activity-dependent neuronal-glial and synaptic plasticity in the adult mammalian hypothalamus. NeuroScience 57, 501–535.

Thomas, L.B., Steindler, D.A., 1995. Glial boundaries and scars: programs for normal development and wound healing in the brain. Neuroscientist 1, 142–154.

Van der Toorn, A., Syková, E., Dijkhuizen, R.M., Vořišek, I., Vargová, L., Škobisová, E., Van Lookeren Campagne, M., Reese, T., Nicolay, K., 1996. Dynamic changes in water ADC, energy metabolism, extracellular space volume, and tortuosity in neonatal rat brain during global ischemia. Magn. Reson. Med. 36, 52–60.

Van Harreveld, A., Dafny, N., Khattab, F.I., 1971. Effects of calcium on electrical resistance and the extracellular space of cerebral cortex. Exp. Neurol. 31, 358–367.

Vargová, L., Chvátal, A., Andìrová, M., Kubinová, Š., Ziak, D., Syková, E., 2001. Effect of osmotic stress on potassium accumulation around glial cells and extracellular space volume in rat spinal cord slices. J. Neurosci. Res. 65, 129–138.

Vargová, L., Poulain, D.A., Oliet, S.H.R., Syková, E., 2002. Changes in diffusion parameters associated with anatomical plasticity of the rat supraoptic nucleus. In: proceedings of the 52nd Annual Meeting of the Society for Neuroscience (abstracts of papers). Orlando.

Vargová, L., Oliet, S.H.R., Syková, E., 2003. Anatomical plasticity-induced changes of extracellular space volume and geometry in rat supraoptic nucleus. In: Proceedings of the Sixth IBRO World Congress of Neuroscience (abstracts of papers). Prague.

Vořišek, I., Syková, E., 1997a. Evolution of anisotropic diffusion in the developing rat corpus callosum. J. Neurophysiol. 78, 912–919.

Vořišek, I., Syková, E., 1997b. Ischemia-induced changes in the extracellular space diffussion parameters, K^+, and pH in the developing rat cortex and corpus callosum. J. Cerebral Blood Flow Metabol. 17, 191–203.

Vořišek, I., Hájek, M., Tintìra, J., Nicolay, K., Syková, E., 2002. Water ADC, extracellular space volume and tortuosity in the rat cortex after traumatic injury. Magn. Reson. Med. 48, 994–1003.

Xie, Y., Zacharias, E., Hoff, P., Tegtmeier, F., 1995. Ion channel involvment in anoxic depolarisation indused by cardiac arrest in rat brain. J. Cerebral Blood Flow Metabol. 15, 587–594.

Yang, P., Yin, X., Rutishauser, U., 1992. Intercellular space is affected by the polysialic acid content of NCAM. J. Cell Biol. 116, 1487–1496.

Zoli, M., Jansson, A., Syková, E., Agnati, L.F., Fuxe, K., 1999. Intercellular communication in the central nervous system. The emergence of the volume transmission concept and its relevance for neuropsychopharmacology. Trends Pharmacol. Sci. 20, 142–150.

Zoremba, N., Homola, A., Lehmenkühler, A., Syková, E., 2003. Extracellular diffusion parameters in rat neocortex during recovery from mild and severe global ischemia. In: Proceedings of the Sixth IBRO World Congress of Neuroscience (abstracts of papers). Prague.