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EXTRASYNAPTIC VOLUME TRANSMISSION AND DIFFUSION PARAMETERS OF THE EXTRACELLULAR SPACE

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Abstract—Extrasynaptic communication between neurons or neurons and glia is mediated by the diffusion of neuroactive substances in the volume of the extracellular space (ECS). The size and irregular geometry of the diffusion channels in the ECS substantially differ not only around individual cells but also in different CNS regions and thus affect and direct the movement of various neuroactive substances in the ECS. Diffusion in the CNS is therefore not only inhomogeneous, but often also anisotropic. The diffusion parameters in adult mammals (including humans), ECS volume fraction α (α=ECS volume/total tissue volume) and tortuosity λ (λ^2=free/apparent diffusion coefficient), are typically 0.20–0.25 and 1.5–1.6, respectively, and as such hinder the diffusion of neuroactive substances and water. These diffusion parameters modulate neuronal signaling, neuron-glia communication and extrasynaptic "volume" transmission. A significant decrease in ECS volume fraction and an increase in diffusion barriers (tortuosity) occur during neuronal activity and pathological states. The changes are often related to cell swelling, cell loss, astrogliosis, the rearrangement of neuronal and astrocytic processes and changes in the extracellular matrix. They are also altered during physiological states such as development, lactation and aging. Plastic changes in ECS volume, tortuosity and anisotropy significantly affect neuron–glia communication, the spatial relation of glial processes toward synapses, glutamate or GABA 'spillover' and synaptic crosstalk. The various changes in tissue diffusivity occurring during many pathological states are important for diagnosis, drug delivery and treatment.

Key words: anisotropy, apparent diffusion coefficient, diffusion-weighted MRI, tortuosity, volume fraction.

Signal transmission in the CNS results from the activation of receptors located in synapses as well as the activation of extrasynaptic receptors. Transmission that does not require synapses and classical receptors is achieved by the accumulation of ions, by the activation of membrane transport processes including uptake and by diffusible signals, e.g. nitric oxide (Fig. 1). Neurons interact both by synapses and by the diffusion of molecules and neuroactive substances in the extracellular space (ECS). Furthermore, neurons release chemical substances not only at the site of their synaptic contacts, but also from the extrasynaptic regions of their membranes. Since glial cells do not have synapses, their communication with neurons is only mediated by the diffusion of ions and neuroactive substances in the ECS, and their release of chemical signals is only non-synaptic. Therefore, both neurons and glia release ions, transmitters and various other neuroactive substances that diffuse through the ECS and bind to extrasynaptic, usually high-affinity, binding sites located on neurons, axons and glial cells. This type of extrasynaptic transmission is also called 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; Kiss and Vizi, 2001; Syková, 2001a; Syková, 2003).

Volume transmission is not a single type of communication (see Fig. 1), and as such it acts as both a short and long distance mode of communication. Its underlying mechanism is the diffusion of neuroactive substances in the ECS, which therefore serves not only as the microenvironment of nerve cells but also as an information channel (Syková, 1997). This type of communication without synapses provides a mechanism for synchronizing neuronal activity and long-range information processing in functions such as vigilance, sleep, lactation, chronic pain, hunger, depression, long-term potentiation (LTP), long-term depression (LTD), memory formation and other plastic changes in the CNS. Many pathological situations accompanied by structural (neuronal and glial) rearrangement as well as changes in the extracellular matrix (ECM) or the deposition of macromolecules, e.g. β amyloid, dramatically affect the ECS diffusion parameters (Mazel et al., 2001; Voříšek et al., 2002a).

Certain synapses ("private synapses") or even whole neurons are clearly tightly ensheathed by glial processes and by the ECM, so-called perineuronal nets (Celio et al., 1998); others are left more "naked." The "open" synapses are easily reached by molecules diffusing in the ECS (Fig. 2). Nevertheless, transmitters, e.g. 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

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Abbreviations: ADC, apparent diffusion coefficient; CSPG, chondroitin sulfate proteoglycan; D, free diffusion coefficient; DW-MRI, diffusion-weighted magnetic resonance imaging; EAE, experimental autoimmune encephalomyelitis; ECM, extracellular matrix; ECS, extracellular space; GFAP, glial fibrillary acidic protein; IOS, intrinsic optical signals; ISM, ion-selective microelectrode; $[K^+]_e$ extracellular potassium concentration; LTD, long-term depression; LTP, long-term potentiation; MRI, magnetic resonance imaging; PSA, polysialic acid; SON, supraoptic nucleus; ST, sulfotransferase; TEA, tetraethylammonium; TMA, tetramethylammonium; TN, tenascin.

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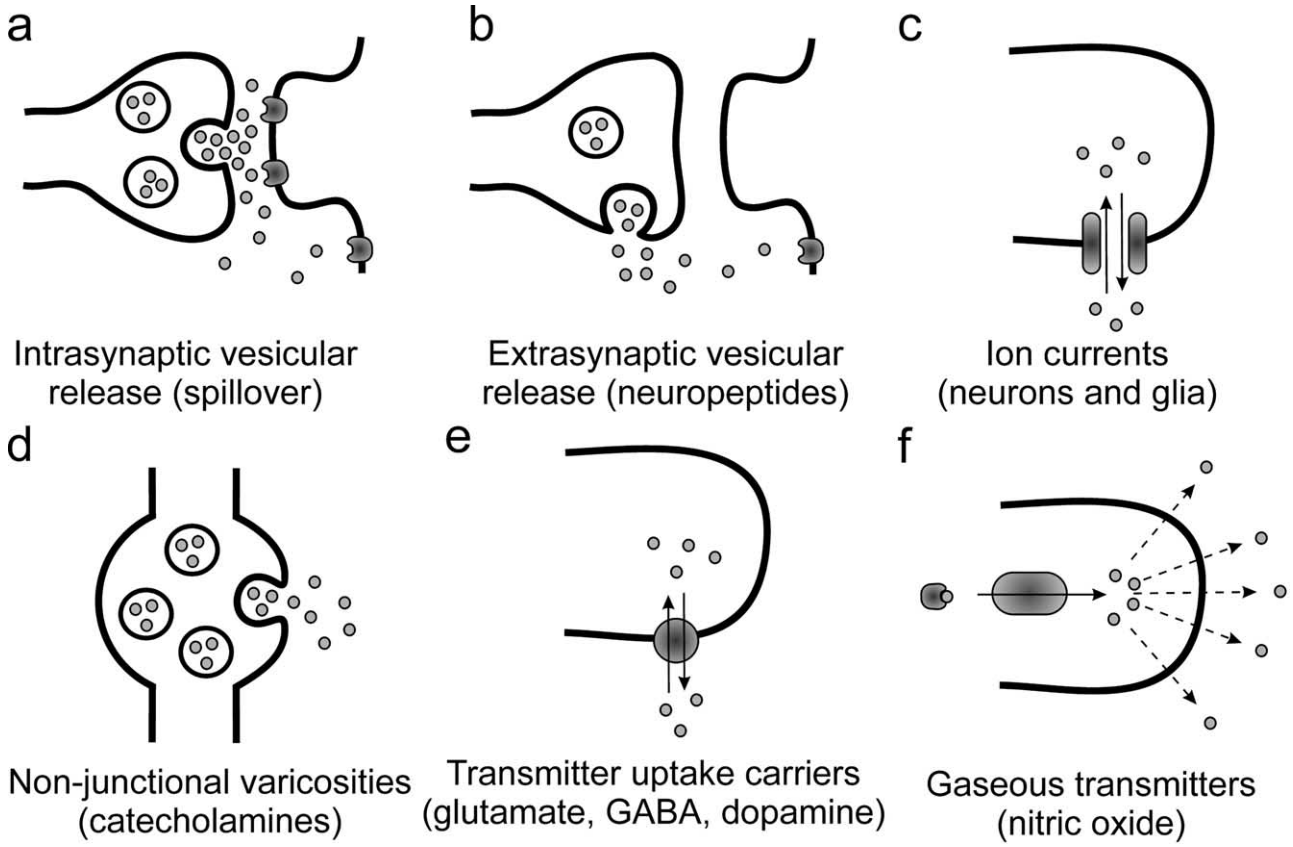

Fig. 1. Schematic representation of the main sources of volume transmission signals in the CNS. (a) Open synaptic transmission: intrasynaptic vesicular release followed by the diffusion of the transmitter outside the synaptic cleft at an effective concentration (synaptic spillover of, e.g. amino acids). (b) Open synaptic transmission: extrasynaptic vesicular release. The transmitter is released directly into the extracellular fluid outside the synaptic cleft (nonsynaptic release of, e.g. neuropeptides). (c) Local ion currents: changes in the extracellular fluid concentration of ions, e.g. K^+, H^+ and Ca^{2+}, induced by the activity of transmitter or voltage-gated ion channels located in neurons or glia. (d) Paracrine transmission: vesicular release from nonjunctional varicosities (for example, diffuse catecholaminergic systems), i.e. varicosities lacking presynaptic specializations and postsynaptic densities. (e) Reverse functioning of transmitter uptake carriers, e.g. the release of glutamate and GABA from astroglia and glutamate and dopamine from neurons. (f) Release of gaseous transmitters, e.g. nitric oxide release from neurons and endothelial cells. (Adapted from Zoli et al., 1999.)

crosstalk" (Kullmann et al., 1996; Asztely et al., 1997). This loss of synaptic independence means that transmitter released at one synapse can lead to the activation of receptors in nearby synapses. The physico-chemical and structural properties of the tissue, glial cells and the ECM may critically influence intersynaptic diffusion. Crosstalk can be particularly relevant during LTP and LTD; it involves glutamate as well as GABA spillover and the activation of iontrophic as well as metabotropic receptors (Isaacson et al., 1993; Kullmann et al., 1996; Piet et al., 2004).

At the level of a single synapse, four elements—the presynaptic and postsynaptic terminals, nearby glial processes and the ECS with its macromolecules—form a unique and plastic entity called a "quadripartite" synapse (Fig. 2). Glial cells maintain not only ECS ionic homeostasis, but also ECS volume homeostasis (by swelling and shrinking due to ionic shifts). In addition, they produce various ECM molecules and, when hypertrophied or proliferating, may form additional diffusion barriers (Syková, 1997, 2001b, 2002). In this way, glial cells can critically affect diffusion anisotropy, tissue permissiveness, synaptic efficacy and extrasynaptic transmission. Glial cells may therefore contribute to short-term plasticity by affecting transmitter spillover by physically, and transiently, ensheathing one synapse but not another (Fig. 2). Furthermore, the diffusing transmitter may not only bind to nearby synaptic receptors, but also to receptors located extrasynaptically, possibly leading to the formation of new dendritic spines.

Diffusion in the ECS

Diffusion is a physical process already well characterized by Einstein (1956) as the random motion of molecules (i.e. Brownian motion) resulting from the thermal energy carried by these molecules. Compared with diffusion in a free medium where molecules move randomly, diffusion in the ECS is critically dependent on, and limited by, the structure and physico-chemical properties of the ECS—the nerve cell microenvironment. The ECS is not homogeneous, since its properties vary not only around each cell, but also in different brain regions. For example, the size of the ECS differs around certain glial cell types, e.g. it is smaller around oligodendrocytes than around astrocytes (Chvátal et al., 1999; Vargová et al., 2001). Moreover, in white as

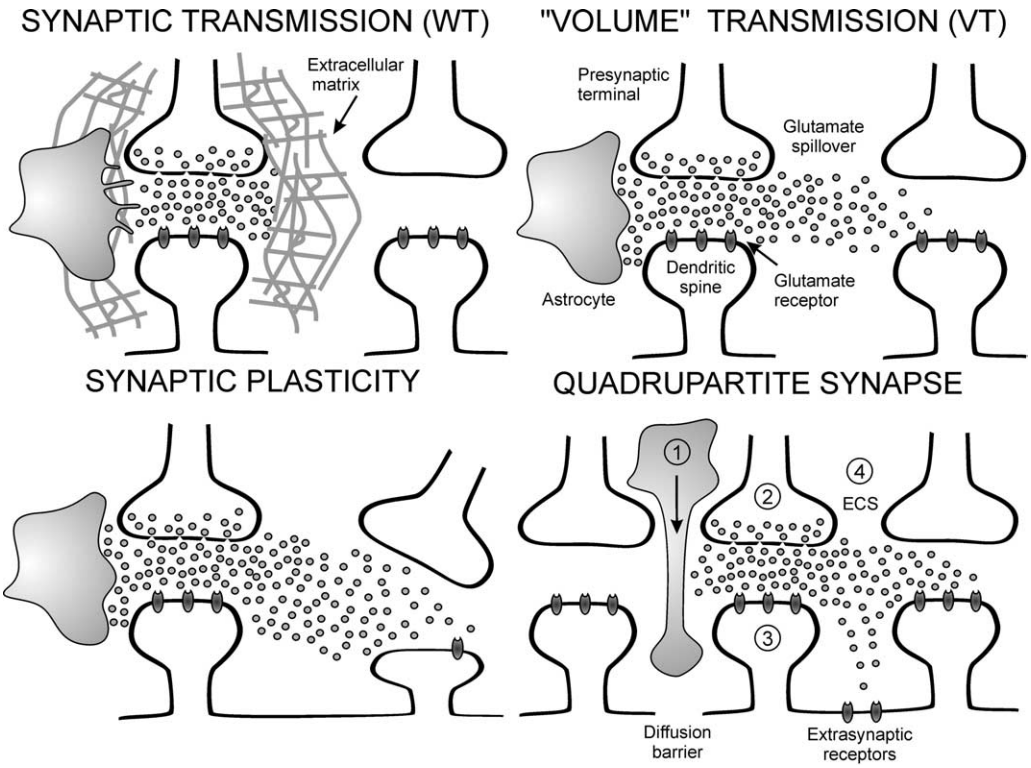

Fig. 2. Concept of synaptic "wired" transmission (WT) and extrasynaptic "volume" transmission (VT). Closed synapses are typical of synaptic transmission. The synapse is tightly ensheathed by glial processes and the ECM, forming perineuronal or perisynaptic nets. An open synapse is typical of volume transmission. It allows the escape of transmitter (e.g. glutamate, GABA) from the synaptic cleft (spillover), diffusion in the ECS and binding to receptors on nearby synapses. This phenomenon is known as 'crosstalk' between synapses. Spillover may also lead to plastic changes, inducing the formation of new synapses or eliciting the rearrangement of astrocytic processes around the synapse and the formation of diffusion barriers. The concept of a quadripartite synapse involves the contribution of four partners-an astrocyte (1), a presynaptic terminal (2), a postsynaptic membrane (receptors on a dendritic spine or extrasynaptic receptors-3), and the ECS (ECS-4). (Adapted from Syková, 2001a.)

well as in gray matter, diffusion is more facilitated in certain directions than in others, e.g. along axons (Rice et al., 1993; Voříšek and Syková,1997a) or along astrocytic processes (Syková et al., 1998, 2002), and therefore we speak about diffusion anisotropy.

The size and irregular geometry of the diffusion channels in the ECS (tissue tortuosity and anisotropy) substantially differ not only around individual cells but also in various CNS regions and thus affect and direct the movement of various neuroactive substances. The diffusion parameters thereby modulate neuronal signaling, neuron–glia communication and extrasynaptic "volume" transmission. They are changed during brain development, glial cell maturation, proliferation, hypertrophy and cell swelling, by changes in ECS ionic composition, the ECM and ECS geometry, and as the result of neuronal loss. They are also altered during lactation, aging, CNS injury, anoxia/ischemia, spreading depression, inflammation, demyelination, in tumors and many other brain physiological or pathological states (Syková and Chvátal, 2000; Syková et al., 2000; Syková, 2001a,b, 2002).

The diffusion of substances in a free medium, e.g. in water or in diluted agar, is described by Fick's laws. In contrast to a 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 ECM, charged molecules and also by cellular uptake (Fig. 3A, B). To take into account these factors, it was necessary to modify Fick's original diffusion equations Nicholson and Phillips, 1981; Nicholson and Syková, 1998). Compared with free solution, 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 the adult brain amounts to about 20–25% of the total brain volume, i.e. $\alpha = 0.20-0.25$. In addition, in tissue the free diffusion coefficient (D) is reduced by the tortuosity factor (λ). ECS tortuosity is defined as $\lambda^2 = D/ADC$, where D is the 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 $ADC = D/\lambda^2$. Thus, any substance diffusing in the ECS is hindered by membrane obstructions, vessels, glycoproteins, macromolecules of the ECM, charged molecules and fine neuronal and glial cell processes (Fig. 3B). Finally, substances released into the ECS are transported across cell membranes (particularly into astrocytes) or across vascular walls by non-specific concentration-dependent uptake (k'). In many cases however, these substances are transported by energy-dependent uptake sys-

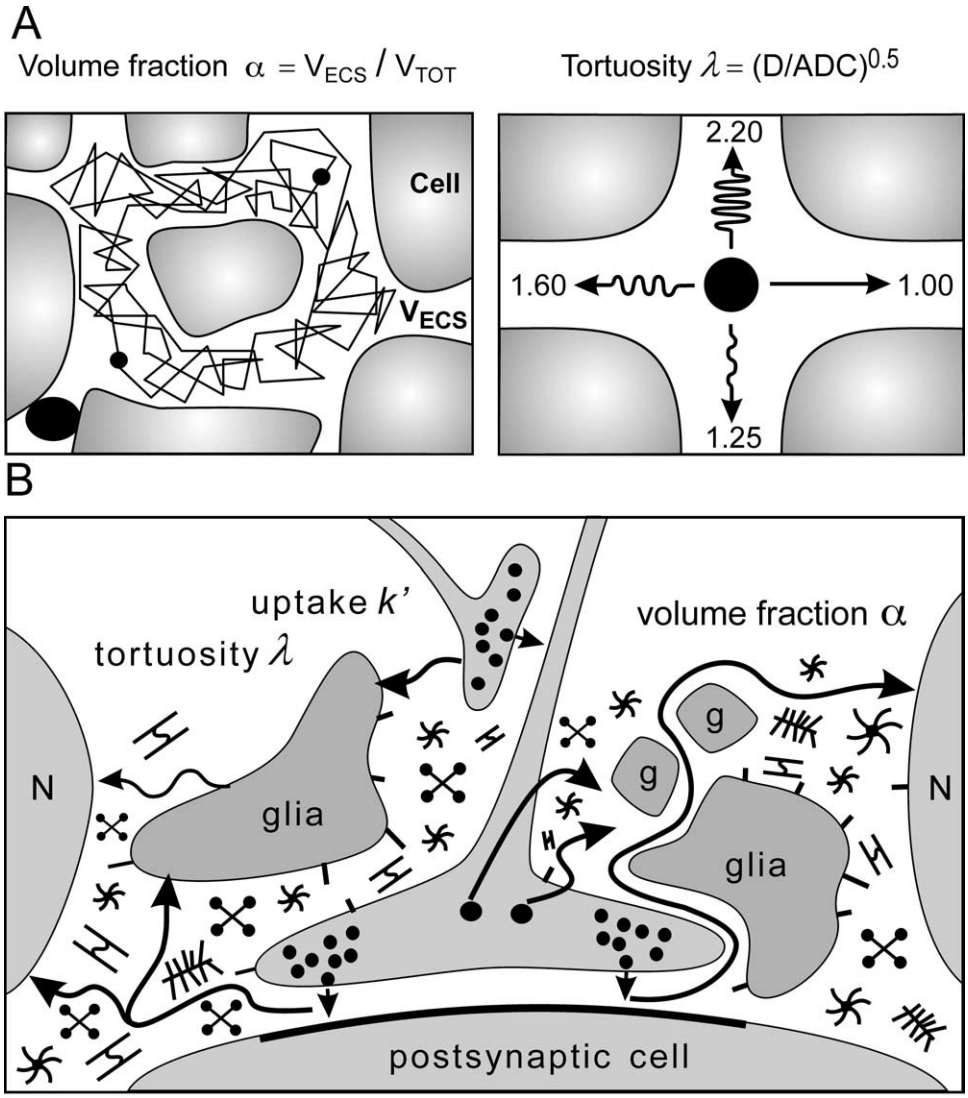

Fig. 3. (A) Diffusion parameters describing the diffusion of a substance through the ECS, volume fraction α ($\alpha = V_{ECS}/V_{TOT}$) and tortuosity λ ($\lambda^2 = D/ADC$). (B) Schematic of CNS architecture. The CNS architecture is composed of neurons (N), axons, glial cells, cellular processes (g), molecules of the ECM 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′*). (Adapted from Syková, 1997.)

tems 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).

Real-time iontophoretic tetramethylammonium (TMA^+) method

The real-time iontophoretic method is the only method used to determine the absolute values of all three ECS diffusion parameters (α, λ and *k′*) and their dynamic changes in nervous tissue *in vitro* as well as *in vivo* (Nicholson and Phillips, 1981; 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^+), TMA^+, choline, or anions such as AsF_6 or α-naphthalene sulfonate (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. 4A). Usually, such electrode arrays are made by gluing together a pressure or iontophoretic pipette and an ISM with a tip separation of 100–300 μm. In the case of the iontophoretic application of TMA^+, the ions are released into the ECS by applying a current step of +80 nA with a duration of 40–80 s. The released ions are recorded with a TMA^+-ISM as a diffusion curve, which is then transferred to a computer for evaluation. Values of α, ADC, λ and *k′* are extracted by a non-linear curve-fitting simplex algorithm operating on the diffusion curve described by equation [1] below, which represents the behavior of TMA^+, assuming that it spreads out with spher-

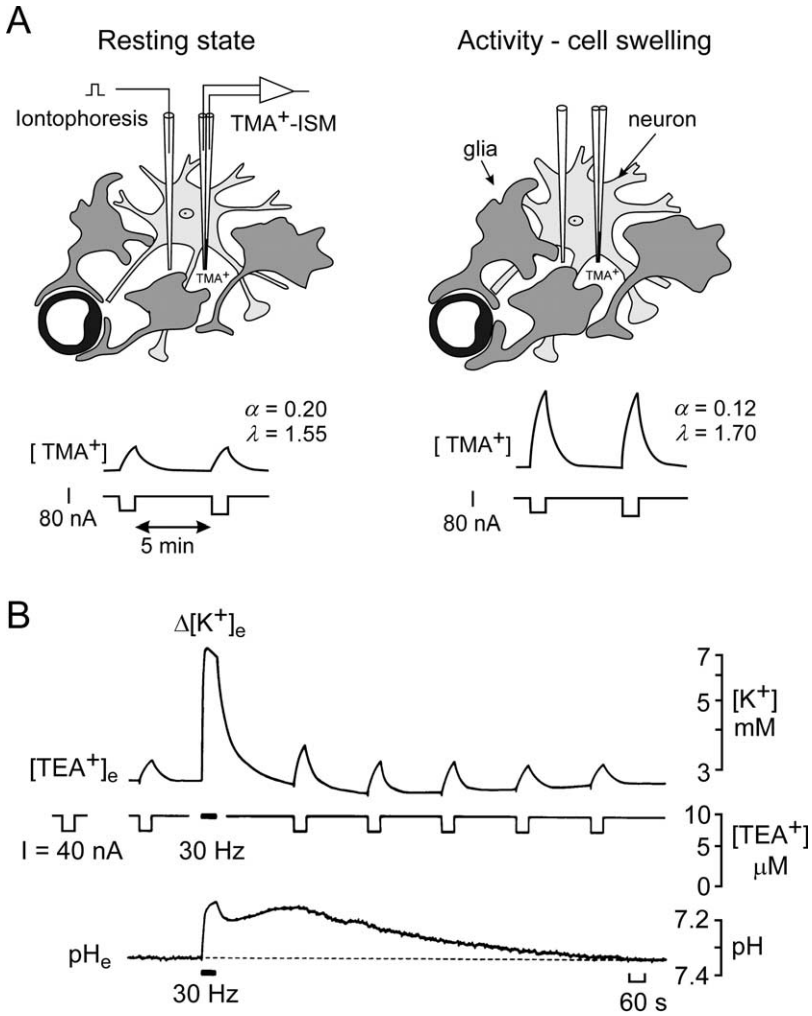

Fig. 4. (A) Experimental setup, TMA^+ diffusion curves and typical ECS diffusion parameters α (volume fraction) and λ (tortuosity) obtained in brain before (left, resting state) and during (right, activity) neuronal activity, evoked by the same iontophoretic current of 80 nA. A TMA^+-selective double-barreled ISM was glued to a bent iontophoresis microelectrode. ECS in unstimulated brain is 20% (volume fraction $\alpha=0.20$) and tortuosity is 1.55. The ECS is smaller due to cell swelling during stimulation-evoked neuronal activity and, therefore, the diffusion curves are bigger. ECS volume decreased to about 12% ($\alpha=0.12$) while λ increased to 1.70. (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 a decrease in α due to cell swelling outlasting the 1 min repetitive stimulation (30 Hz) of the sciatic nerve for 30 min. Note the extracellular K^+ increase and acid shift evoked by stimulation. The time course of the acid shift correlates with the decrease in α. (Adapted from Syková, 1992, 1997.)

ical 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:

$C=G(t)t<S$, for the rising phase of the curve

$C=G(t)-G(t-S)t>S$, 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 (Nicholson and Phillips, 1981):

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

The quantity of TMA^+ or TEA^+ delivered to the tissue per second is $Q=In/z\mathrm{F}$, where I is the step increase in current applied to the iontophoresis electrode, n is the transport number, z is the number of charges associated with the substance iontophoresed ($+1$ for TMA^+ or TEA^+), and F is 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.

Other methods used to study ECS diffusion parameters

The other methods used so far to study ECS volume fraction and geometry *in vivo* have been less comprehensive, since either they can only give information about relative changes in the ECS volume fraction, or these changes are only partly related to cell swelling and ECS

shrinkage and some other, often unknown mechanisms, can contribute to these signals. These methods include measurements of tissue resistance (Van Harreveld et al., 1971; Matsuoka and Hossmann, 1982; Korf et al., 1988), changes in light transmittance and/or scattering (MacVicar and Hochman, 1991; Andrew et al., 1996, 1999; Fayuk et al., 2002; Tao et al., 2002; Syková et al., 2003), measurements of the ADCs of molecules tagged with fluorescent dye (Nicholson and Tao, 1993; Prokopová-Kubinová et al., 2001) and measurements of the ADC of water molecules (ADC_W) using diffusion-weighted magnetic resonance imaging (DW-MRI). In many of our studies, we compared two methods in animal models of pathological states and related the results to morphological changes determined by immunohistochemical methods.

The concept of DW-MRI is to produce MRI-based quantitative maps of the microscopic, natural displacement of water molecules that occurs in brain tissues as part of the physical diffusion process (Le Bihan, 2003). Diffusion-weighted MRI methods (Benveniste et al., 1992; Latour et al., 1994; Norris et al., 1994; Van der Toorn et al., 1996; Sotak, 2004) give information about ADC_W. The relationship between water movement, ADC_W maps and changes in cell volume and ECS diffusion parameters (α and λ) is not yet well understood. It is evident that similarly as the diffusion of small substances such as TMA^+, water diffusion in the brain is also inhomogeneous and anisotropic (Basser et al., 1994; Pierpaoli et al., 1996; Vořišek et al., 2002b; Mamata et al., 2002). In ADC_W maps we can identify different brain regions that correspond to those recognized histologically. Comparing the TMA^+ method with DW-MRI, we have found that decreases in ADC_W can be related either to decreases in α (Van der Toorn et al., 1996) or to increases in λ without apparent changes in α (Vořišek et al., 2002b).

Recordings of intrinsic optical signals (IOS), either light transmittance or light reflectance, are believed to reflect changes in the ECS volume fraction; however, the evidence is not compelling. In fact, direct evidence is missing, since under a number of experimental conditions the relationship does not exist (Syková et al., 2003; Jarvis et al., 1999; Tao et al., 2002). Simultaneous recordings of light transmittance, α and extracellular K^+ in rat spinal cord slices during electrical stimulation and the application of elevated potassium, NMDA or anisosmotic solutions revealed substantial differences (Fig. 5). The application of 6 or 10 mM K^+ or NMDA (10^{-5} M) had no measurable effect on α, 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^+. Moreover, while the change in α persisted for many minutes, the light transmittance changes quickly returned to control levels and decreased below them. On the other hand, the elevation of extracellular K^+ after NMDA application, corresponding to increased neuronal activity, had a similar time course as did the light transmittance changes. The results of these experiments provide considerable evidence that light transmittance changes do not result simply from water shifts across cellular membranes, i.e. from changes in cell volume, but are strongly associated with neuronal activity resulting in fine morphological changes in neurons and astrocytes (Syková et al., 2003).

In the mammalian CNS, the water channel AQP4 is concentrated in astrocyte endfeet membranes facing the blood–brain and brain–cerebrospinal fluid interfaces. The AQP4 channel was shown to have a role in the formation of brain edema and in brain water homeostasis. Some studies indicate that down-regulation of AQP4 may protect against water influx during the onset of acute brain edema, but may also prolong the edematous state by interfering with brain water efflux during the resolution phase (Amiry-Moghaddam et al., 2003). It was also shown that regional differences in APQ4 levels and distribution matched differences in the rate of development of brain edema as assessed by DW-MRI. ADC_w showed a more abrupt decrease in the cerebellum than in the neocortex or striatum. This suggests that water influx occurs more rapidly in the cerebellum than in the other structures investigated (Amiry-Moghaddam et al., 2004). The question remains whether AQP4 may affect extracellular diffusion parameters under physiological conditions. In experiments performed in neocortical slices, Niermann et al. (2001), using IOS measurements correlated with changes in extracellular K^+, found that vasopressin affects water flux in the neocortex mediated through the AQP4-containing astrocytic syncytium. This corresponds to our experiments showing that IOSs are related to increases in extracellular K^+ due to enhanced neuronal activity (Syková et al., 2003). However, it remains to be determined how AQP4-related water shifts contribute to cell swelling and compensatory ECS volume changes under more physiological conditions.

Diffusion anisotropy

The diffusion of molecules and neuroactive substances 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 preferentially channels the movement of substances, including water, in the ECS in one direction (e.g. along axons in the corpus callosum) and is, therefore, responsible for a certain degree of specificity in volume transmission. Using the TMA^+ method, the ECS diffusion parameters α, $\lambda_{x,y,z}$ and k' were measured in the cortex, corpus callosum, cerebellum, hippocampus (CA1, CA3 and in dentate gyrus) and spinal cord of rats and mice *in vivo*. If diffusion in a particular brain region is anisotropic, then the correct value of α cannot be calculated from measurements done only in one direction. The correct value of α can be determined (Rice et al., 1993; Mazel et al., 1998) only if TMA^+ diffusion is measured in the ECS independently along three orthogonal axes (*x*, transversal; *y*, sagittal; *z*, vertical).

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 (Pérez-Pinzon et

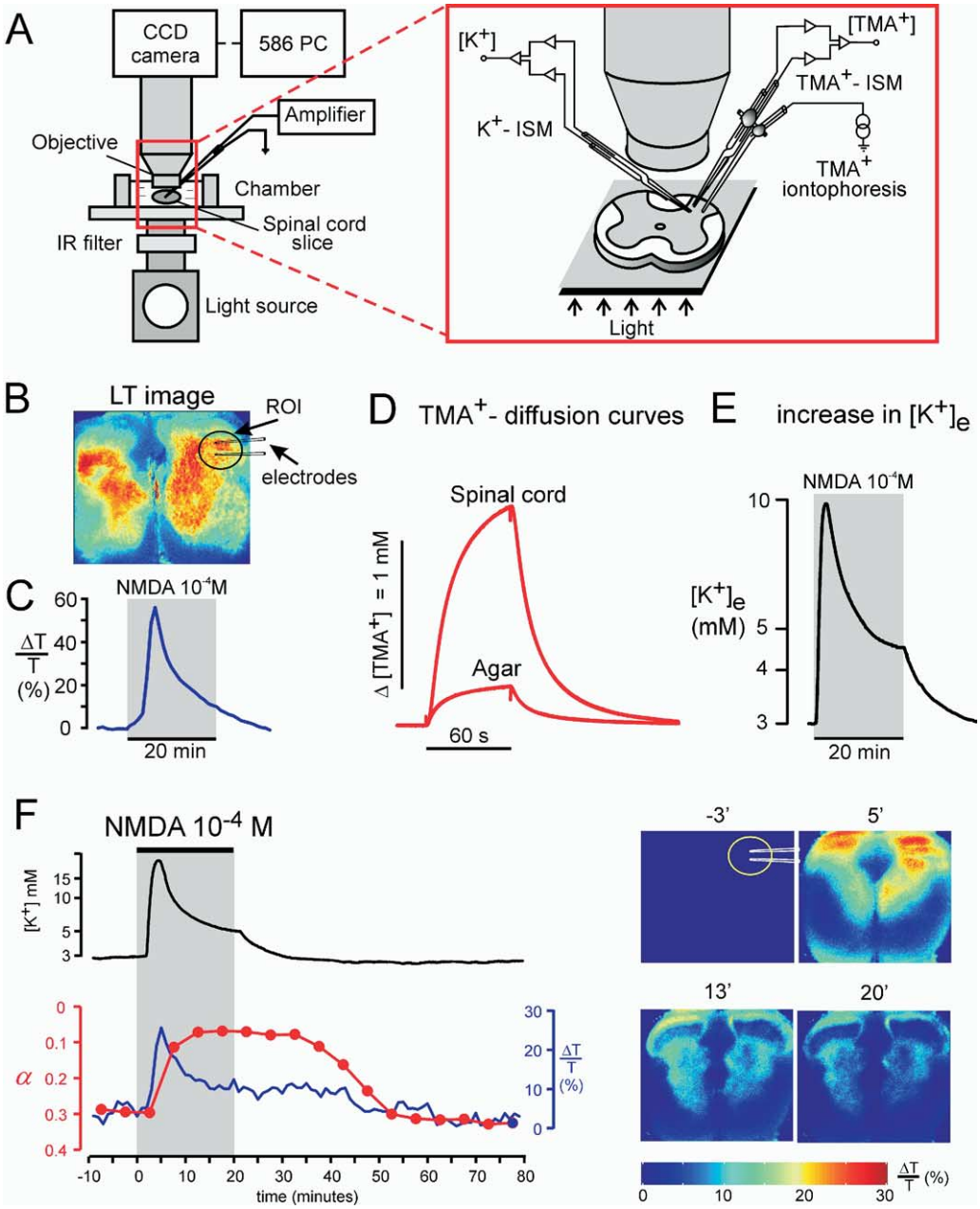

Fig. 5. Experimental arrangement for the simultaneous measurement of light transmittance, ECS diffusion parameters and/or changes in extracellular K^+ concentration ($[K^+]_e$). (A) ISM were inserted into the dorsal horns of spinal cord slices and fixed by a U-shaped platinum wire with a nylon grid in a perfusion chamber mounted on the stage of a fluorescence microscope. (B) Example of changes in light transmittance (LT) evoked by a 20 min application of 10^{-4} M NMDA. The pseudocolor image reflects the spatial distribution of the percentage of change in light transmittance 5 min after the onset of the application. The time course of light transmittance changes, expressed as the percentage of change ($\Delta T/T\%$) in the area of the dorsal horn selected as the region of interest (ROI), is shown in C. (D) Typical recordings of TMA^+ diffusion curves in agar and in spinal cord gray matter during perfusion with artificial cerebrospinal fluid. (E) Typical recording of an increase in $[K^+]_e$ evoked by 10^{-4}M NMDA application. (F) Simultaneous measurements of extracellular potassium concentration ($[K^+]$), TMA^+ diffusion parameters and light transmittance in spinal cord slices (400 μm) of a 14-day-old rat. Changes in α (ECS volume fraction) before, during and after perfusion of the slice with 10^{-4} M NMDA were extracted by appropriate non-linear curve fitting and plotted with the same time course as the light transmittance changes (IOS). Right: IOS images taken 3 min before application ($-3'$; control), and at 5, 13, and 20 min after the application of NMDA for 20 min. Cellular, particularly glial, swelling results in a decrease in α. While the changes in TMA diffusion parameters persist during and after the application period, the changes in IOS decrease with time. It is also evident that the time course of the IOS signal peaks faster than the changes in ECS volume. The changes in IOS, however, correspond to the increase in extracellular $[K^+]$, which in turn corresponds to an increase in neuronal activity. There is, therefore, a good correlation of IOS with neuronal excitation, while no simple correlation exists between the IOS signal and ECS volume changes. (Adapted from Syková et al., 2003.)

al., 1995; Mazel et al., 1998; Fig. 6), and in the auditory but not in the somatosensory cortex (Syková et al., 1999). Other studies revealed that anisotropy is present in the myelinated white matter of the corpus callosum or spinal

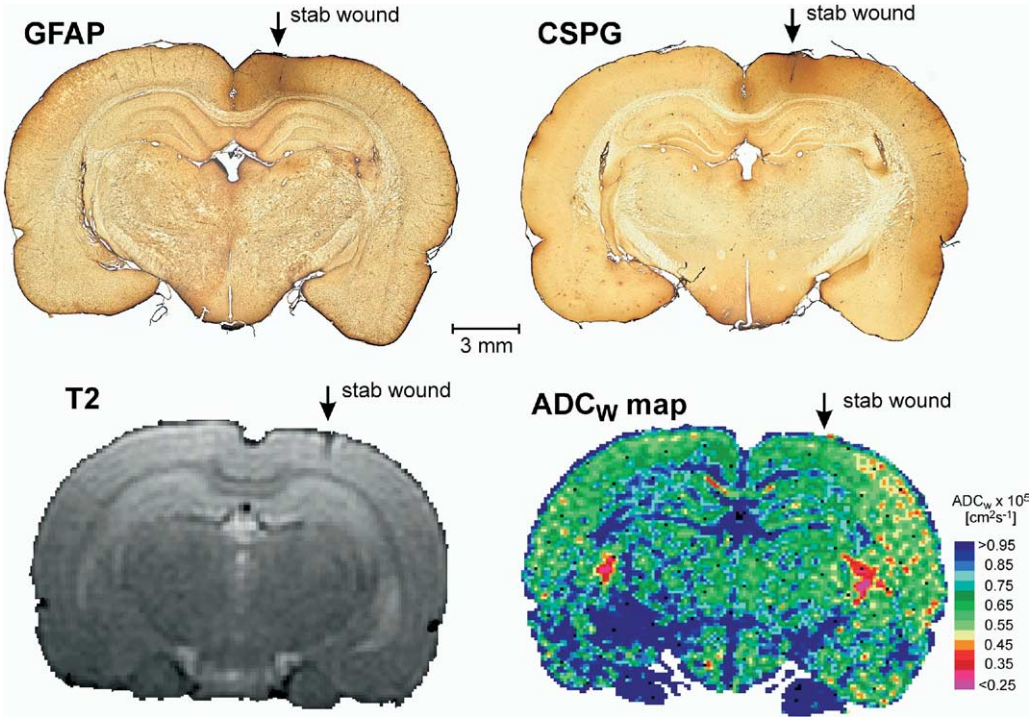

Fig. 6. Coronal rat brain sections stained for GFAP (upper left) and CSPG (upper right) 7 days following a cortical stab wound. The results show a higher level of GFAP expression in the vicinity of the stab wound and a higher level of CSPG expression in the whole cortex of the injured hemisphere. Both the GFAP and CSPG sections are from the same animal. The arrows indicate the site of the stab wound. Lower left: T2-weighted image showing the localization of the wound. Lower right: A pseudocolor image showing a typical ADC_W map of an injured rat brain 7 days post-wounding; ADC_W was measured along the *y*-axis (rostrocaudal plane). The scale to the right of 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 with control animals, except in the area close to the wound. (Adapted from Vořišek et al., 2002b.)

cord white matter (Vořišek 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 (Vořišek and Syková, 1997a; Prokopová et al., 1997).

Glia and ECS diffusion parameters

Ions as well as neurotransmitters released into the ECS during neuronal activity or pathological states interact not only with the 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 α, particularly the swelling and possible rearrangement 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.

Transmembrane ionic fluxes during neuronal activity are accompanied by the movement of water and cellular, presumably particularly glial, swelling. Changes in ECS diffusion parameters (α decrease, λ increase and ADC decrease) are the consequence of activity-related transmembrane ionic shifts and cellular swelling. In the spinal cord of the rat or frog, repetitive electrical stimulation results in a rise in extracellular potassium concentration ($[K^+]_e$) by 4–6 mM and in a decrease in α from about 0.24–0.12-0.17, i.e. α decreases by as much as 30–50% (Fig. 4B; Syková, 1987; Svoboda and Syková, 1991). After injury of the ipsilateral hind paw evoked by a s.c. injection of turpentine or after thermal injury, $[K^+]_e$ increases, pH_e decreases and α in the spinal dorsal horn also decreases by 20–50%. 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 (Svoboda and Syková, 1991).

Changes in α contribute to the mechanism of nonspecific feedback suppressing neuronal excitability. 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 (Fig. 4B). 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 α leads to a greater accumulation of ions and neuroactive substances,

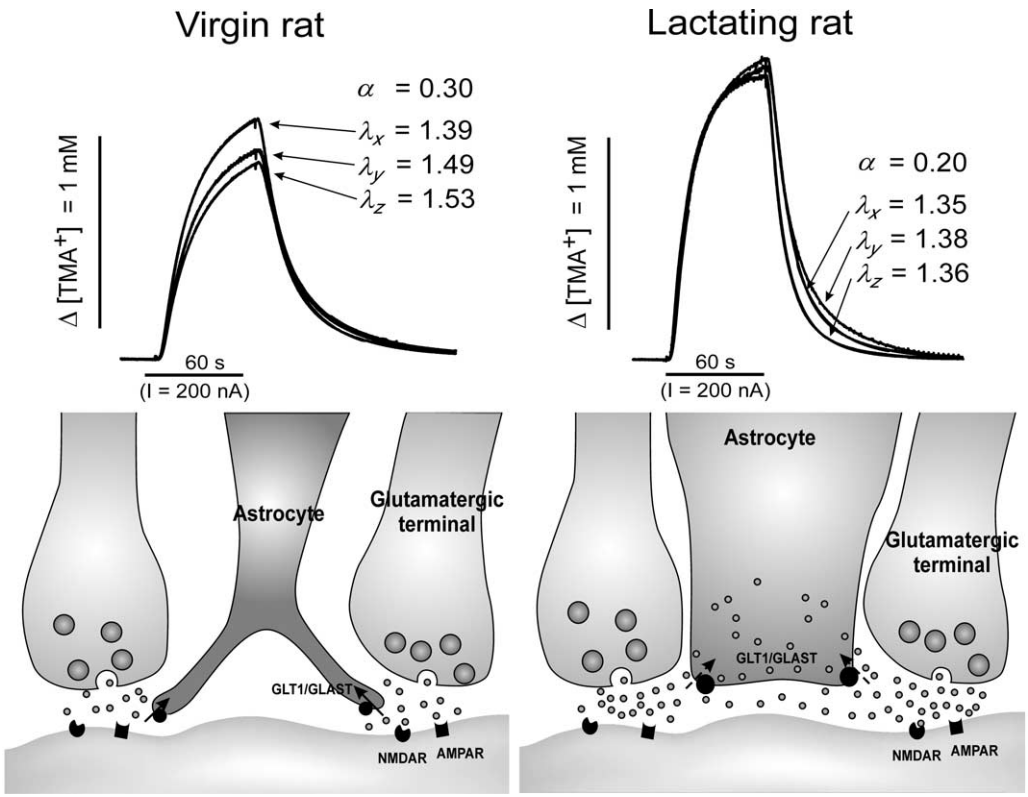

Fig. 7. Scheme of the hypothesized anatomical remodeling (retraction of astrocytic processes) occurring in the rat SON during lactation. Reduced astrocytic coverage of SON neurons in lactating rats leads to deficient glutamate clearance, resulting in increased glutamate concentration in the ECS, increased crosstalk between synapses and increased activation of either presynaptic or postsynaptic receptors. Examples of diffusion curves obtained in virgin (upper left) and lactating (upper right) rats. Values for tortuosity (λ) and volume fraction (α) extracted from three curves recorded along the *x*-, *y*- and *z*-axes are indicated. (Adapted from Piet et al., 2004.)

the "crowding" of molecules of the ECM in the ECS and decreases in the ADCs of various molecules.

Many pathological processes in the CNS are accompanied by a loss of cells or neuronal processes, astrogliosis, demyelination, and changes in the ECM, all of which may affect the ADCs of neuroactive substances. Several animal models have been developed to study changes in the ECS diffusion parameters. Brain injury of any kind elicits reactive gliosis, involving both hyperplasia and hypertrophy of astrocytes, which show intense staining for glial fibrillary acidic protein (GFAP). Astrogliosis is also a typical characteristic of cortical stab wounds in rodents (Norton et al., 1992). Using the TMA method to measure ECS diffusion parameters in a stab wound lesion, we found an increase in α and a substantial increase in λ to mean values of about 0.26 for α and about 1.8 for λ (Roitbak and Syková, 1999a). Severe astrogliosis was found close to the wound, mild astrogliosis in the ipsilateral but not the contralateral cortex (Fig. 6: GFAP). Chondroitin sulfate proteoglycan (CSPG) expression was, however, increased throughout the whole ipsilateral cortex (Fig. 6: CSPG). In the hemisphere contralateral to the wound, α, λ as well as ADC_W, measured in the same animal by DW-MRI, were not significantly different from control values. α 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. However, λ increased and ADC_W decreased after lesioning, both in the vicinity of the wound as well as in the rest of the ipsilateral hemisphere distant from the wound (Fig. 6: ADC_W map). Thus, both ADC_W and ADC_{TMA} decreased in regions with no change in α, but where CSPG expression increased (for details, see the role of the ECM below). An increase in ECM expression may therefore impose diffusion barriers not only for TMA^+ molecules but also for water.

The swelling of glial cells due to water shifts accompanying large K^+ and pH_e changes occurs 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 increases and pH_e begins to decrease by about 0.1 pH unit. With the further fall in blood pressure, pH_e decreases to pH 6.4–6.6. This pH_e decrease is accompanied by a steep rise in $[K^+]_e$ up to about 50–70 mM and decreases in $[Na^+]_e$ to 48–59 mM, $[Cl^-]_e$ to 70–75 mM and $[Ca^{++}]_e$ to 0.06–0.08 mM. Together with the ionic changes there is a DC slow potential shift, an accumulation of excitatory amino acids such as glutamate, cell swelling and a compensatory decrease in α, which starts to decrease when the blood pressure drops below 80 mm Hg and $[K^+]_e$ rises above 6 mM (Syková et al., 1994). Ultimately, the ECS decreases to about 20–25% of its normal volume ($\alpha = 0.04-0.07$).

During hypoxia and terminal anoxia, α in rat cortex or spinal cord decreases from about 0.20 to about 0.05, while λ 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 gray and white matter, and 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 (Voříšek and Syková, 1997b). This corresponds to incomplete gliogenesis and to the well-known resistance of the immature CNS to anoxia.

Structural changes during lactation-astrocytes and ECS plasticity

There is growing evidence that transmitters do not always remain sequestered within the synaptic cleft, but can diffuse and exert their action at distant receptors located extrasynaptically and even on adjacent synapses (Barbour and Häusser, 1997; Rusakov et al., 1999). Such interactions between independent synaptic inputs, known as synaptic crosstalk, have been shown in several brain regions where synaptically released glutamate induces the inhibition of GABAergic and glutamatergic transmission through the activation of presynaptic metabotropic, kainate or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (Mitchell and Silver, 2000; Semyanov and Kullmann, 2000). These forms of heterosynaptic modulation depend on the extent of neurotransmitter diffusion into neighboring synapses. Such extrasynaptic regulatory processes are likely therefore to be influenced by the surrounding astrocytes, which not only ensure most transmitter uptake through high affinity transporters (Schousboe, 2003), but also represent a physical barrier to diffusion in the ECS (Nicholson and Syková, 1998; Roitbak and Syková, 1999; Syková, 2001).

In the supraoptic nucleus (SON), 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 ECS diffusion parameters in acute slices from virgin and lactating rats. Measurements were performed along three perpendicular axes (*x*, *y* and *z*) in the SON using the real-time TMA^+ iontophoretic method (Piet et al., 2004). The values of both λ and α were reduced in lactating animals, showing that the retraction of astrocytic processes (reduced astrocytic coverage) resulted in enhanced diffusion (Fig. 7). These findings indicate that the SON is an anisotropic structure in which 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. Thus, the plastic changes occurring in the SON during lactation facilitate the diffusion of neuroactive substances. Under these conditions glutamate spillover, which was monitored through the metabotropic glutamate receptor-mediated depression of GABAergic transmission (LTD), was greatly enhanced (Piet et al., 2004).

Our studies of the heterosynaptic depression of evoked inhibitory postsynaptic currents measured in the SON of lactating rats revealed that the ECS parameters directly influence the mGluR-mediated depression of GABA release by regulating the action of glutamate at a distance from its release sites. Because glial cells in the SON in virgin rats represent a physical barrier to diffusion and are essential for glutamate uptake, the retraction of their processes could result in glutamate-mediated crosstalk between synapses (Fig. 7). Indeed, we found that the reduction of the astrocytic coverage of SON neurons dramatically favors diffusion in the tissue and, as a consequence, glutamate-induced heterosynaptic depression of GABAergic transmission (Piet et al., 2004). On the other hand, this form of intersynaptic crosstalk was largely prevented when diffusion in the ECS was limited by the introduction of a large neutral molecule, such as dextran, into the ECS. These data, therefore, indicate that the astrocytic environment of neurons is a critical regulator of communication between neighboring synapses, and, probably, of overall extrasynaptic transmission in the CNS.

Role of ECM molecules

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 (e.g. tenascins, TN) and proteoglycans that constitute the ECM. Various ECM molecules and adhesion molecules have been described, e.g. fibronectin, tanescin, laminin, etc. (Thomas and Steindler, 1995; Celio et al., 1998), and their amount 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 Figs. 2 and 3, these large molecules can slow down the movement (diffusion) of various neuroactive substances through the ECS.

TN are a family of large extracellular glycoproteins. Whereas TN-R is an important component of the adult brain's ECM, TN-C is expressed mainly during early development, while HNK-1 is a sulfated carbohydrate epitope that attaches to these molecules and modifies their adhesive properties. In order to assess the influence of these three molecules on the ECS diffusion parameters, we used the TMA method to measure α and λ and diffusion-weighted MRI to measure the apparent diffusion coefficient of water (ADC_W). All measurements were done *in vivo* in the cortex of TN-R, TN-C or HNK-1 sulfotransferase (ST) deficient mice and their wild-type littermate controls. The lack of TN-R or HNK-1 ST resulted in a significant decrease in α, λ and ADC_W. Compared with controls, α in TN-R $-/-$ and in ST $-/-$ mice decreased by 22% and 9%, respectively. In TN-C $-/-$ animals no significant changes were observed in comparison to controls. We concluded

that in TN-R and HNK-1 ST deficient mice, which show morphological, electrophysiological and behavioral abnormalities, ECS size and geometry are altered. TN-R, as an important component of the ECM, appears to maintain an optimal distance between cells. A compromised ECS affects the diffusion of neuroactive substances in the brain and may alter neuron–glia interactions, synaptic efficacy and extrasynaptic transmission (Antonova et al., 2001; Chvátal and Syková, 2002).

Modification of the ECM can also be achieved by enzymatic treatment. Hyaluronic acid and CSPGs are essential components of the ECM, 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 abundant 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 sulfate 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 or chondroitinase ABC and studied the acute (3 h) and chronic (24 h) effects of this treatment on ECS diffusion parameters. A significant decrease in λ was already found in the ipsilateral hemisphere 2 h after injection and persisted at 24 h (Syková, 2001a). Although there was a small increase in α in the first 2–3 h after injection, a slight decrease in α below control values was found at 24 h. On the other hand, the incubation of isolated rat spinal cord with 0.1% hyaluronic acid increases λ in the spinal dorsal horns (Syková, 2003).

The role of the ECM in determining ECS volume can also be demonstrated by the changes seen during development. Early postnatally, an elevated ECM molecule content is accompanied by high values of α (Lehmenkühler et al., 1993; Voříšek and Syková, 1997a). The decreased intensity of immunohistochemical staining for CSPG and fibronectin observed in aged animals coincides with a decrease in α in these animals compared with young adults (Syková et al., 1998). A similar correlation is also seen in many pathological states, e.g. astrogliosis, in which both an increased production of ECM molecules and an increase in α have been observed (Syková et al., 1999; Roitbak et al., 1999). The explanation for this correlation is simple: the high density of negative charges carried by many ECM molecules, especially glycosaminoglycans, attracts osmotically active cations, such as Na^+, causing large amounts of water to be drawn into the matrix. This creates a swelling pressure, or turgor, that enables the matrix to withstand compressive forces and leads to the expansion of the ECS (Alberts et al., 1994). Thus, the loss of ECM during aging might lead to a decrease in α. On the other hand, the deposition of β-amyloid in aged transgenic APP23 mice, in which overproduction of mutated human amyloid precursor protein leads to plaque formation and memory impairment, is accompanied by a significant increase in α. ECM molecules may therefore act like a sponge by binding a large number of water molecules and, due to the mutual repulsion of their numerous negatively charged residues, tend to occupy a lot of space.

ECS volume changes during pathological states

Changes in ECS diffusion parameters have been found in all brain and spinal cord pathologies studied to date. Besides anoxia/ischemia (described above), these changes accompany many demyelinating, inflammatory, degenerative and malignant diseases. In chronic disease models, α and λ often behave as independent variables, their changes being related to structural changes resulting from the reaction of glial cells as well as neurones to pathological processes. This often leads also to the loss of diffusion anisotropy and therefore to the loss of a certain degree of volume transmission specificity. Human inflammatory demyelinating diseases, e.g. multiple sclerosis, are characterized by the 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 α in all regions of the spinal cord, namely in the dorsal horn (from 0.21–0.28), ventral horn (from 0.23–0.47), the intermediate region (from 0.22–0.33) and in white matter (from 0.18–0.30). The enlarged α was accompanied by a decrease in λ and a decrease in k'. 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 α 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.

Tumor cell migration through the ECS has been shown to be affected by the ECS volume fraction and ECM molecule content. The diffusion parameters in temporal cortical tissue resected during the surgical treatment of temporal lobe epilepsy (control) were compared with those in brain tumors (Vargová et al., 2003). Subsequently, tumor slices were histopathologically classified according to WHO grading, and proliferative activity was assessed. The average values of α and λ in control cortex were 0.24 and 1.55, respectively. The values of α and λ in oligodendro-

gliomas did not significantly differ from controls. In pilocytic astrogliomas (WHO grade I) as well as in ependymomas (WHO grade II), α was significantly higher while λ was not changed. Higher values of α as well as λ were found in low-grade diffuse astrocytomas (WHO grade II) and in cellular regions of high-grade astrocytomas (WHO grade III and IV). Tumor malignancy grade strongly corresponded to an increase in α, which was accompanied by a change in ECS structure manifested as an increase in diffusion barriers for small molecules (Vargová et al., 2003). In this study, we found a positive correlation between increasing values of α and proliferative activity in each distinct histological group of investigated tumors. We can therefore speculate that the malignancy of primary brain tumors and their proliferative and migratory activity may also be dependent on the ability of the tumors to create space for migrating cells by the destruction of the surrounding tissue. It has been shown that the over-expression of certain ECM molecules correlates with increased tumor malignancy and migratory activity (Camby et al., 2001; Hayen et al., 1999; Zhang et al., 1998), since these molecules may serve as "tracks" and thus facilitate tumor cell migration. However, they may form diffusion barriers for neuroactive molecules, including chemotherapeutics, the diffusion of which will be constrained.

ECS diffusion parameters during aging

Aging, Alzheimer's disease and many degenerative diseases are accompanied by serious cognitive deficits, particularly impaired learning and memory loss. This functional decline in old age is a consequence of changes in brain anatomy, morphology and volume. Nervous tissue, particularly in the hippocampus and cortex, is subject to 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 ECM proteins, etc. (for review, see Grady and Craik, 2000). These and other changes not only affect the efficacy of signal transmission at synapses, but could also affect extrasynaptic transmission.

Volume fraction is decreasing during the entire postnatal life with the steepest decrease in early postnatal development (Voříšek and Syková, 1997a; Lehmenkühler et al., 1993). The larger ECS (30–45%) in the first days of postnatal development in the rat can be attributed to incomplete neuronal migration, gliogenesis, angiogenesis and to the presence of large ECM 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 from each other.

Using the TMA^+ method, α, $\lambda_{x,y,z}$ and k' were measured in the cortex, corpus callosum and hippocampus (CA1, CA3 and in the dentate gyrus) of aged rats. In all the studied regions—cortex, corpus callosum and hippocampus—the mean α was significantly lower in aged rats (26–32 months old), ranging from 0.17–0.19, than in young adults (3–4 months old) in which α ranged from 0.21–0.22. From Fig. 8 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. 8). This means that there is a loss of anisotropy in the aging hippocampus, particularly in the CA3 region and the dentate gyrus (Syková et al., 1998).

Morphological changes during aging include cell loss, the loss of dendritic processes, demyelination, astrogliosis, swollen astrocytic processes and changes in the ECM. 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 ECM (Syková et al., 1998). Astrogliotic changes can explain why α in the cortex, corpus callosum and hippocampus of senescent mice and rats is significantly lower than in young adults. 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 α (Syková et al., 1998). Other changes could account for the decreases in λ values and for the disruption of tissue anisotropy. In the hippocampus in the CA1, CA3, as well as in the dentate gyrus, we observed changes in the arrangement of fine astrocytic processes. These are normally organized in parallel in the *x*–*y* plane, but this organization totally disappears during aging. Moreover, decreased staining for CSPG and for fibronectin suggests a loss of ECM macromolecules, which can contribute to a decrease in α.

Our study also revealed that the degree of learning deficit during aging correlates with the changes in α, λ and k' (Syková et al., 2002). The hippocampus is well known for its role in memory formation, especially declarative memory. 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 (Mazel et al., 1998; Wieshmann et al., 1999). There was a significant difference between mildly and severely behaviorally impaired rats (rats were tested in a Morris water maze), which was particularly apparent in the hippocampus. The ECS in the dentate gyrus of severely impaired rats (bad learners) was significantly smaller than in mildly impaired rats (good learners). Also, anisotropy in the hippocampus of bad learners, particularly in the dentate gyrus, was much reduced, while a substantial degree of anisotropy was still present in aged rats with good learning performance. 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 et al., 1998; Syková, 2001b,, 2002).

The ensuing decrease in α could be explained by the disappearance of a significant part of the ECS matrix, neuronal migration and the development of dendritic trees,

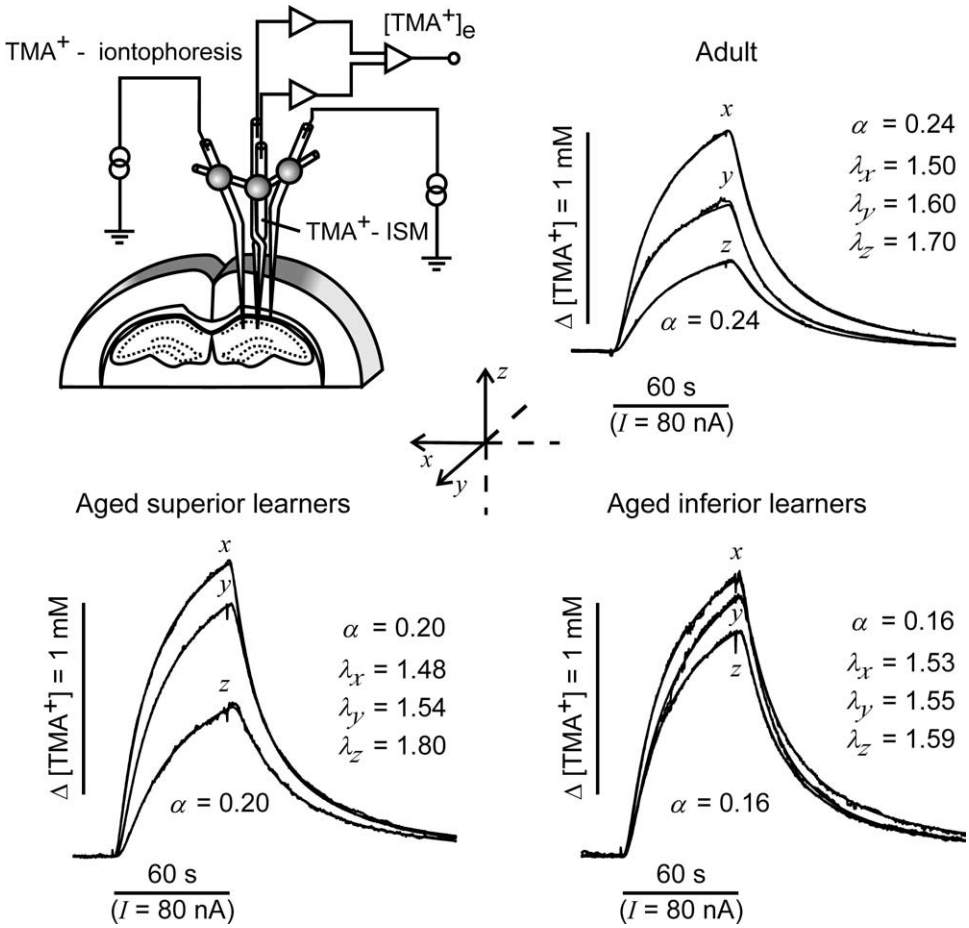

Fig. 8. Experimental setup and typical diffusion curves in the hippocampus. Upper left: Schema of the experimental arrangement; a TMA^+-selective double-barreled microelectrode was glued to two iontophoresis microelectrodes to allow for simultaneous measurements in the *x*- and *y*-axes. Upper right: Anisotropic diffusion in the dentate gyrus of an adult rat; TMA^+ diffusion curves (concentration-time profiles) were measured along three orthogonal axes (*x*, mediolateral; *y*, rostrocaudal; *z*, dorsoventral). The slower rise in the *z* than in the *y* direction and in the *y* than in the *x* direction indicates a higher λ and more restricted diffusion. The amplitude of the curves shows that TMA^+ concentration is much higher along the *x*-axis than along the *y*-axis and even higher than along the *z*-axis. This can be explained if we realize that TMA^+ concentration decreases with the "diffusion distance" from the iontophoretic micropipette, and that the real "diffusion distance" is not *r* but λ*r*. Note that the actual ECS volume fraction α is about 0.24 and can be calculated only when measurements are done in the *x*-, *y*- and *z*-axes. Lower left: Anisotropy still persists in an aged rat that showed superior learning; diffusion curves are higher than in the adult rat shown in the upper right, showing that α is smaller. Lower right: Anisotropy is almost lost in an aged inferior learner. (From Syková et al., 2002.)

rapid myelination and the proliferation of glia. Some of these processes are also observed during aging. The most important are probably neuronal degeneration, a further loss of ECM and astrogliosis. Indeed, we observed a decrease in fibronectin and CSPG staining in the hippocampus of mildly impaired aged rats and almost a complete loss of staining in severely impaired aged rats (Syková et al., 2002). CSPG participate in multiple cellular processes (Hardingham and Fosang, 1992; Margolis and Margolis, 1993). These include axonal outgrowth, axonal branching and synaptogenesis, which are important for the formation of memory traces. The observed loss of anisotropy in senescent rats could therefore lead to impaired cortical and, particularly, hippocampal function. The decrease in α 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.

CONCLUSION

The movement of neuroactive substances in the CNS is affected by water shifts between the intra- and extracellular compartments of the nervous tissue. Diffusion is also hindered by structural changes including the rearrangement of glial processes and the production of ECM molecules which, in addition to cell swelling, is an accompanying phenomenon of many physiological changes and pathological processes. The changes in ECS diffusion parameters affect the efficacy of synaptic as well as extrasynaptic transmission, enhance or suppress neuronal activity, and may induce damage to nerve cells by the increased accumulation of toxic substances. These changes are important for activity-related short-term plasticity, which affects transmitter spillover, crosstalk between synapses and the activation of extrasynaptic receptors on neurons and glia. ECS volume changes are also important for cell migration

in tumors, during cell implantation and subsequent migration as well as when considering therapeutic drug application. Moreover, changes in diffusion parameters may serve as an important indicator of pathological processes, and as such diffusion-weighted MRI is important for diagnostic purposes.

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