L. F. Agnati, K. Fuxe, C. Nicholson and E. Syková (Eds.)
Progress in Brain Research, Vol 125

CHAPTER 9

Glial influence on neuronal signaling

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Introduction

Glial cells, non-excitable cells in the central nervous system (CNS), have been the focus of interest for many investigators since the last century, when they were first observed and described morphologically. Systematic study of the electrophysiological properties of glial cells, astrocytes and oligodendrocytes, began during the 1960s, when Kuffler et al. (1966) first observed that glial cells are depolarized by neuronal activity and thus formulated a possible role for glial cells in the buffering of K^+, which accumulates in the extracellular space of the CNS. Astrocytes, oligodendrocytes and neurons develop from the same type of progenitor cell, and extensive experimental research done over more than 30 years has revealed that glial cells do not play only a passive supporting role in the CNS but have distinct electrophysiological properties, which are determined by the presence of membrane proteins, i.e. by different types of ionic channels and transporters in the cell membrane. Activation of ion channels on glial cells may also lead to the activation of second messengers and intracellular metabolic pathways, and to changes in their volume, particularly swelling and rearrangement of processes, accompanied by dynamic variations in the ECS volume (Syková, 1997). Glial cells may thus, by regulating their volume, influence extracellular pathways for neuroactive substances and affect extrasynaptic 'volume' transmission in the CNS. There is increasing evidence that another type of glial cell found in the CNS, microglial cells, also plays an important role in the normal as well as the pathological brain and can have both neurotrophic and neurotoxic properties (Eder, 1998). In contrast to neurons, astrocytes and oligodendrocytes, microglial cells develop from bone marrow-derived monocytes and invade the brain during embryonic development. Since a number of comprehensive reviews describing the membrane properties of glial cells have already been published (Walz, 1989; Chesler, 1990; Barres, 1991; Sontheimer, 1994; Gallo and Russell, 1995; Steinhäuser and Gallo, 1996; Oh, 1997; Porter and McCarthy, 1997; Verkhratsky et al., 1998; Eder, 1998), the present chapter focuses on the relation between the electrophysiological properties of glial cells in the brain in situ, the properties of the extracellular space and synaptic and volume transmission in the CNS.

Membrane properties of glial cells in situ

The first studies of the electrophysiological properties of glial cells were performed in tissue cultures (Hild and Tasaki, 1962). Since it was soon apparent that cultured glial cells, that develop in an environment lacking neurons, may express a wide variety of voltage- and ligand-activated currents, the question arose whether the properties of the glial membrane in culture match the properties of glial

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membrane in vivo (for review see Porter and McCarthy, 1997). The first attempt to avoid cell culture artifacts was made by developing the technique of freshly isolating glial cells from brain tissue, when the membrane properties of glial cells were studied from different regions of the nervous tissue, e.g. optic nerve or hippocamus (Barres et al., 1990; Tse et al., 1992). By applying the patch-clamp technique to thin brain slices (Edwards et al., 1989), it became possible to investigate glial cells in their normal cellular environment and to re-evaluate the results obtained in tissue culture and by other techniques. Furthermore, brain slices permit the study of glial cells during development, since slices from animals of different postnatal ages can be compared. During the past decade of extensive research on the electrophysiological properties of glial cells in situ by means of patch-clamp technique, the interest of investigators has been predominantly focused on the following areas of the mammalian CNS: corpus callosum, hippocampus, cerebellum and spinal cord. It was found that glial cells studied in brain slices may express, like neurons, a number of voltage- and ligand-activated channels. In contrast to neurons, a large variability in the current patterns among glial cell types is observed in response to depolarizing and hyperpolarizing voltage steps (Fig. 1). Such stimulation may activate Na^+ channels, delayed-rectifier K^+ channels (K_{DR}), A-type K^+ channels

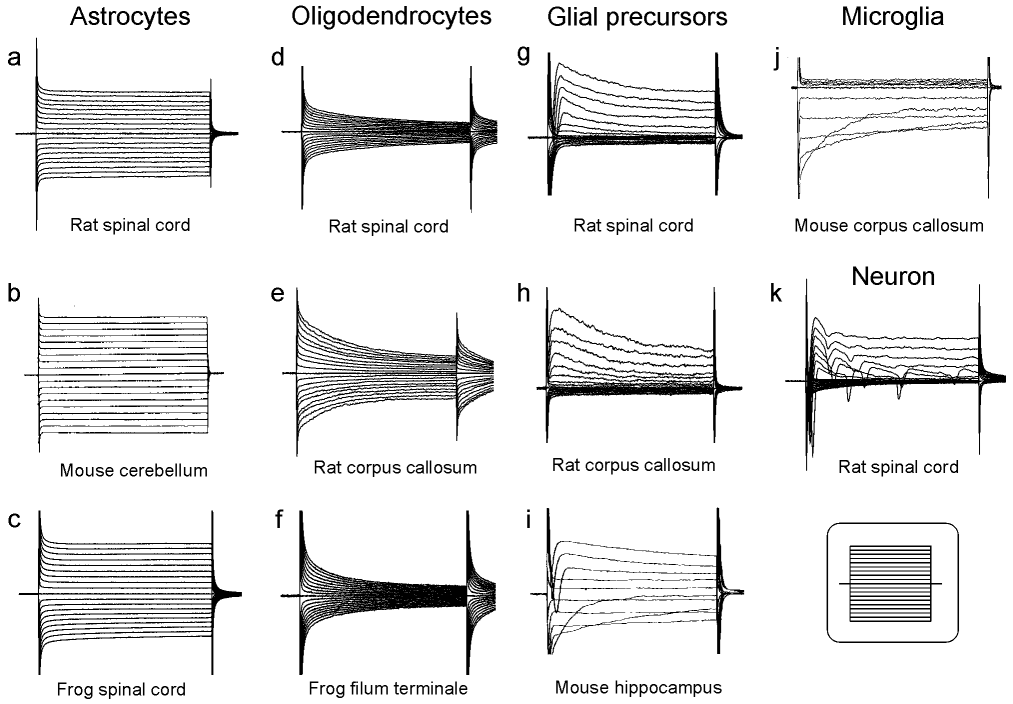


Fig. 1. Typical membrane current patterns of astrocytes (a, b, c), oligodendrocytes (d, e, f), glial precursor cells (g, h, i), microglial cells (j) and neurons (k) evoked by depolarizing and hyperpolarizing voltage steps in different areas of the brain in situ. Membrane currents were recorded while the membrane potential was clamped at values close to –70 mV. To activate voltage-gated currents, the membrane was clamped for 50 ms to increasing de- and hyperpolarizing potentials (pattern of voltage commands in inset) ranging from –160 mV to +20 mV with 10 mV increment. Current traces are not corrected for leakage and capacitance currents. Mature astrocytes and oligodendrocytes express passive currents, while glial precursors, neurons and microglia cells express various types of voltage-activated currents. Current traces in (b) adapted from Kirischuk et al., 1996; current traces in (e, h) adapted from Chvátal et al., 1997; current traces in (i) adapted from Steinhäuser et al., 1992; current traces at (j) adapted from Brockhaus et al., 1993; current traces in (a, c, d, f, g, k) are unpublished data.

(K_A), inward rectifier K^+ channels (K_{IR}) and Ca^{2+} channels. A combined approach of patch-clamp technique, Ca^{2+} imaging and confocal microscopy revealed that glial cells in situ respond to the application of glycine, GABA, glutamate, ATP, histamine, norepinephrine, serotonin, angiotensin II, bradykinin, and substance P (Fig. 2) and that the ionotropic effect of some ligands in glial cells is different from that described in neurons (Fig. 3).

Astrocytes and oligodendrocytes in the spinal cord gray matter

In the gray matter of rat spinal cord slices, astrocytes, oligodendrocytes and their respective precursors can be distinguished at postnatal days 1 to 19 (P1–19), based on their pattern of membrane currents and their morphological and immunocytochemical features (Chvátal et al., 1995). Astrocytes are identified by positive staining for glial fibrillary acidic protein (GFAP), while oligodendrocytes are identified by a typical oligodendrocyte-like morphology, a lack of GFAP staining, and positive labeling with O1 or O4 antibodies – markers of the oligodendrocyte lineage. Electrophysiologically, mature astrocytes are characterized by symmetrical, non-decaying K^+-selective currents. The membrane of mature oligodendrocytes exhibits symmetrical passive, but decaying K^+ currents with prominent tail currents (I_{tail}) after the offset of the voltage command (see also Chvátal et al., 1999a). Astrocyte precursor cells express a complex pattern of voltage gated channels, namely Na^+, K_{DR}, K_A and K_{IR} channels. In comparison to those of neurons, the amplitude of these Na^+ currents is at least one order of magnitude less; none of these cells show the ability to generate action potentials in the current clamp mode. Oligodendrocyte precursors are distinguished by the presence of K_{IR}, K_{DR}, and K_A. Studies performed on glial cells in spinal cord slices from GFAP-positive and GFAP-negative mice revealed a similar distribution of voltage-activated currents in astrocytes, oligodendrocytes and glial precursor cells as was described in the rat spinal cord (Anděrová et al., 1999). The same pattern of voltage-activated membrane currents was also observed in preliminary studies performed on glial cells in frog spinal cord slices (Anděrová, Chvátal, Syková, unpublished results).

Astrocytes, glial precursor cells, and a subpopulation of oligodendrocytes in the gray matter of the rat spinal cord respond to glycine, GABA and glutamate (Pastor et al., 1995). The involvement of glycine receptors is inferred from the

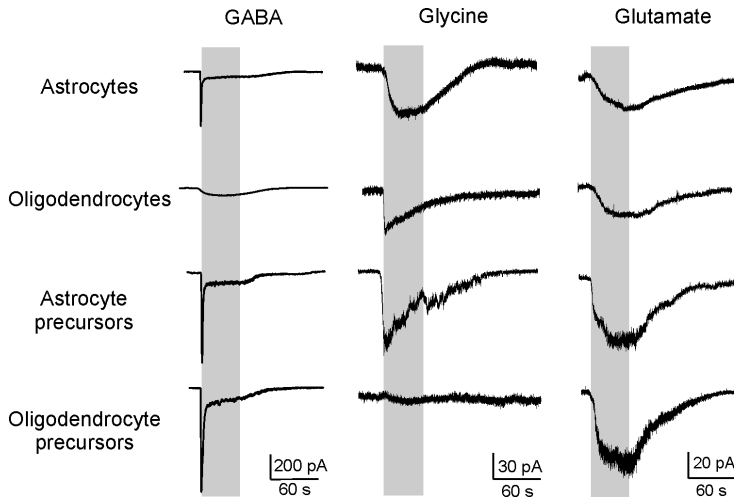


Fig. 2. Membrane currents evoked by GABA (10^{-3} M), glycine (10^{-3} M) and glutamate (10^{-3} M) shown for the four types of glial cells in gray matter of the rat spinal cord slice. Gray bars indicate the time of application of the test substance. Note the difference in the calibrations for the ligand-activated currents. Adapted from Pastor et al., 1995 and Žiak et al., 1998.

observation that the response to glycine is blocked by strychnine and that the induced current reverses close to the Cl^- equilibrium potential. Also, in experiments in which patch-clamp technique was combined with reverse transcription-mediated PCR analysis, the expression of glycine receptor subunits was detected in glial cells in situ (Kirchhoff et al., 1996). GABA-induced membrane currents are mimicked by muscimol and blocked by bicuculline and are large in astrocytes and precursor cells, while oligodendrocytes show only small responses. $GABA_A$ receptor activation also decreases the resting K^+ conductance. All glial cells in the spinal cord gray matter are sensitive to glutamate, kainate, and NMDA (Žiak et al., 1998). Kainate evokes larger currents in glial precursors than in astrocytes and oligodendrocytes, while NMDA induces larger currents in astrocytes and oligodendrocytes than in precursors. Kainate-evoked currents are blocked by the AMPA/kainate receptor antagonist CNQX and are, with the exception of precursors, larger in the dorsal than in the ventral horn, as are NMDA-evoked currents. Currents evoked by NMDA are unaffected by CNQX, are not sensitive to Mg^{2+} and are present when synaptic transmission is blocked in a Ca^{2+}-free solution. NMDA-evoked currents are not abolished during the block of K^+ inward currents in glial cells by Ba^{2+}; thus they are unlikely to be mediated by an increase in extracellular K^+ during neuronal activity.

Oligodendrocytes and microglial cells in corpus callosum

In the mouse corpus callosum, where the first tight-seal patch-clamp recordings from glial cells in situ

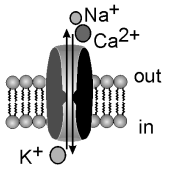
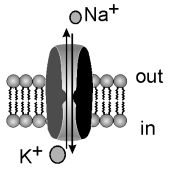
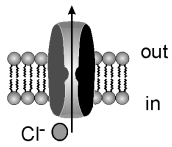
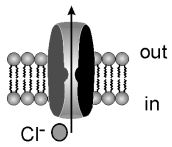
receptor	region of CNS	effect
AMPA /kainate	hippocampus cerebellum spinal cord retina	
NMDA	hippocampus cerebellum spinal cord retina cortex	
$GABA_A$	hippocampus cerebellum spinal cord retina	
glycine	spinal cord	

Fig. 3. Effects of the activation of AMPA/kainate, NMDA, GABA and glycine receptors in situ as described in different regions of the CNS. Partially based on the data in Porter and McCarthy, 1997.

were performed by Berger et al. (1991), more than 99% of all perikarya belong to glial cells during the early postnatal period (Sturrock, 1980). The morphological investigation of these cells, as studied by Lucifer yellow injection, revealed that at P5 numerous thin processes extend radially from the perikaryon of these cells, while at P10–13 most processes extend parallel to each other, thus showing the typical oligodendrocyte-like morphology. In slices from P6–8, oligodendrocyte precursors were found that predominantly expressed K_{DR}, while K_A or Na^+ currents, described in oligodendrocyte precursors in culture, were either absent or only rarely observed. On the other hand, oligodendrocyte precursors observed in a rat corpus callosum preparation (Chvátal et al., 1997) were characterized by the presence of K_A, K_{DR} and K_{IR}. Mature oligodendrocytes in both species are characterized by large passive currents (with an almost linear current-voltage relationship), which decay during the voltage command, and by large I_{tail} after the offset of the voltage pulse.

Oligodendrocyte precursor cells, as well as mature oligodendrocytes, express GABA and glutamate receptors (Berger et al., 1992). GABA-evoked currents show similarities to those described for $GABA_A$ receptors, i.e. they are mimicked by muscimol, blocked by bicuculine and enhanced by pentobarbital. Glutamate-evoked currents show similarities to those of the kainate/quisqalate receptor subtype; oligodendrocyte precursors in the rat corpus callosum express weakly Ca^{2+}-permeable AMPA receptors (Berger, 1995). Kainate in these cells induces two effects: the activation of a cationic current and the block of K^+ conductance. It has also been shown in studies, in which fluorescent dyes were used concomitantly with confocal microscopy to measure intracellular Ca^{2+} transients, that glial cells from mouse corpus callosum slices respond to a variety of neuroligands (Bernstein et al., 1996). Subpopulations of glial cells obtained at P3–7 responded with an intracellular Ca^{2+} signal to the application of ATP, glutamate, histamine, GABA, norepinephrine, serotonin, angiotensin II, bradykinin and substance P, while glial cells obtained from animals at P11–18 responded only to glutamate, ATP and norepinephrine.

Microglial cells in corpus callosum slices from P6–9 mice express very small currents during hyperpolarizing voltage steps in situ, while hyperpolarization induces large inward currents (Brockhaus et al., 1993). Since the reversal potential of these currents depends on the transmembrane K^+ gradient, inactivation time constants decrease with hyperpolarization, and the currents are blocked by tetraethylammonium, the inward currents observed in microglial cells exhibit properties of the inwardly rectifying K^+ channel.

Glial cells in hippocampus

The first patch-clamp recordings from glial cells in the stratum radiatum of the hippocampus were done by Steinhäuser et al. (1992) in brain slices from P10–12 mice. The authors did not distinguish between astrocytes and oligodendrocytes; nevertheless, based on the current pattern, glial cells were grouped into four types. The first is characterized by passive and symmetrical K^+ currents activated both in depolarizing and hyperpolarizing directions. The second population of glial cells expresses a similar current pattern, but with a marked decay of the current during voltage jumps and with a prominent I_{tail} after the offset of the voltage jump. In the third population the decaying passive currents are superimposed with a K_{DR} and, in some cases, with T-type and L-type Ca^{2+} channels, while the fourth population of glial cells expresses K_{DR}, K_{IR} and, in some cases, K_A and Na^+ currents (see also Kressin et al., 1995; Akopian et al., 1996). In another study performed on hippocampal slices of P5–24 rats, glial cells were identified as astrocytes and oligodendrocytes by staining for GFAP (Sontheimer and Waxman, 1993). Astrocytes express K_{DR}, K_{IR}, K_A and Na^+ currents (see also Bordey and Sontheimer, 1997), while oligodendrocytes express either time-independent currents with linear current/voltage properties or time- and voltage-dependent K^+ currents characterized by inward rectification.

In mouse hippocampal slices, the application of GABA evokes inward currents in all types of glial cells (Steinhäuser et al., 1994). There is no apparent difference in the amplitude and time course of GABA-activated currents between GFAP-positive

and GFAP-negative cells. The specific $GABA_A$ receptor agonist muscimol evokes inward currents with similar kinetics and amplitudes as does GABA. In addition, the GABA response in glial cells is reversibly blocked by the $GABA_A$ receptor antagonist bicuculline. The application of glutamate and kainate induces inward currents in all glial cell types in the CA1 stratum pyramidale of the mouse hippocampus, while the application of NMDA induces inward currents only in cells with passive K^+ currents (Steinhäuser et al., 1994). Glutamate increases cation conductance and the blockade of the resting K^+ conductance. It was shown in a study performed in the CA1 stratum radiatum of the mouse hippocampal slice that application of kainate or AMPA also leads to the activation of cationic conductance and to a long-lasting blockade of voltage-gated K^+ channels and, in addition, to an elevation of cytosolic Ca^{2+} (Jabs et al., 1994; Seifert and Steinhäuser, 1995). Similarly, in the hilus of the dentate gyrus of the rat hippocampus, Backus and Berger (1995) showed that glutamate, kainate and AMPA evoked inward currents in astrocytes and presumed glial precursor cells. AMPA receptors in this study were permeable to Ca^{2+}. The electrophysiological data were confirmed by experiments in which confocal microscopy and fluorescent dyes were used in rat hippocampal slices to show that GFAP-positive astrocytes located in the stratum oriens or stratum radiatum from CA1 respond to the perfusion of glutamate, kainate and NMDA (Porter and McCarthy, 1995) or to glutamate released from synaptic terminals (Porter and McCarthy, 1996).

Bergmann glial cells in cerebellum

Bergmann glial cells, an astrocyte-type glial cell in the cerebellum, are closely associated with neurons. During development they provide guiding structures for migrating granule cells; in the adult cerebellum they display intimate interactions with Purkinje cells (Berger et al., 1995; see also Grosche et al., 1999). In Bergmann glial cells from P5–7 mice, K_{DR} and K_{IR} are present, while the same cells in older animals are characterized by large, voltage and time-independent K^+ currents (Müller et al., 1994).

The application of GABA induces a rapid activation of Cl^- conductance and a long-lasting decrease in the resting K^+ conductance. Both effects are mediated by benzodiazepine-insenstitive $GABA_A$ receptors. GABA-evoked currents in cells of P5–7 mice are large compared to the small or even undetectable currents in P20–30 cells. Glutamate, kainate and NMDA induce inward currents in Bergmann glial cells (Müller et al., 1992, 1993). The AMPA/kainate receptor is characterized by a high Ca^{2+} permeability and a sigmoidal current-voltage relationship. The influx of Ca^{2+} blocks the resting K^+ current and the junctional conductances of the cells (Müller et al., 1996). NMDA-induced inward currents are not accompanied by a Ca^{2+} influx and are not affected by the presence of Mg^{2+} or potentiated by glycine.

It was also shown using a combined patch-clamp and confocal microscopy approach, that Bergmann glial cells in situ express metabotropic glutamate receptors, purinergic receptors, α_1-adrenoreceptors and H_1 histamine receptors (Kirischuk et al., 1995, 1996).

Membrane properties of glial cells are determined by their developmental stage

During the last decade of electrophysiological investigations of glial currents in brain slices in situ from different areas of the brain of different species, it has become evident that the membrane properties of glial cells are not uniform, but depend on the developmental stage of the cell (for review see also Berger et al., 1995). For example, astrocyte precursors in spinal cord and in hippocampus are characterized by the presence of Na^+ currents, while in astrocyte-like Bergmann glia these currents have never been detected. Similarly, in oligodendrocyte precursors in the rat spinal cord and corpus callosum, Na^+ currents are not detected, but they are present in mouse corpus callosum slices. On the other hand, all immunohistochemically identified mature astrocytes and oligodendrocytes are characterized by large passive K^+ currents.

In studies of the developmental regulation of ligand-activated currents in glial cells during the first 2–3 postnatal weeks, two approaches have

been used. The first compares precursor vs. mature glial cells. In comparison to mature astrocytes and oligodendrocytes, glial precursor cells are as a rule characterized by significantly larger currents evoked by GABA, kainate and glycine (astrocyte lineage only in the spinal cord). Surprisingly, NMDA-evoked currents are larger in mature astrocytes and oligodendrocytes in the spinal cord (Žiak et al., 1998), in astrocyte-like glial cells in the hippocampus (Steinhäuser et al., 1994), and in Bergmann glial cells in the cerebellum than in glial precursor cells (Müller et al., 1993). The second approach, which so far has been applied only in spinal cord slices, follows the developmental regulation of the ligand-evoked currents within distinct populations of glial cells, i.e. glial precursor cells, mature astrocytes and mature oligodendrocytes (Pastor et al., 1995; Žiak et al., 1998). GABA-evoked currents are down-regulated only in astrocytes, while in the other cell populations they do not significantly change. Glycine-evoked currents significantly decrease only in glial precursor cells, while kainate-evoked currents do not change during development in any glial population and NMDA-evoked currents significantly decrease in glial precursor cells as well as mature astrocytes and oligodendrocytes.

It is possible to conclude that during the first 2–3 weeks of postnatal development, the occurrence of voltage-activated currents in glial cells decreases, while the passive K^+ conductance rapidly increases. Similarly, ligand-activated currents either do not change or decrease during the transition from glial precursor to mature glial cell as well as during early postnatal development within each glial cell population.

Glial membrane properties are affected by regional differences in ECS volume

Glial cells as well as neurons in the CNS are surrounded by the extracellular space (ECS), which creates their natural microenvironment. The composition and properties of the ECS are established during development, but the most dramatic changes in the composition of the ECS are observed during neuronal activity, when a number of neuroactive substances are released from neurons into the ECS, e.g. ions, transmitters, peptides, neurohormones and metabolites, which diffuse via the ECS to their targets located on nerve as well as glial cells, distant from the release sites (for reviews see Syková, 1997; Nicholson and Syková, 1998; Zoli et al., 1999). The ECS serves as a communication channel between cellular elements in the CNS. In addition to classical synaptic transmission, substances move by diffusion through the volume of the ECS and this type of signal transmission is called extrasynaptic or volume transmission (Agnati et al., 1995; Syková, 1997; Nicholson and Syková, 1998; Zoli et al., 1999). Properties of the ECS and their changes during neuronal activity, development, aging and many pathological states, affect the diffusion of substances in the ECS and may thus affect neuronal signaling and neuron-glia communication (see Chapter 6 by E. Syková et al.). Extrasynaptic transmission is altered by diffusion barriers that may be formed by fine glial and neuronal processes as well as by adhesion molecules and molecules of the extracellular matrix. It was also shown in recent studies that such diffusion barriers exist around oligodendrocytes and that these regional differences in the ECS affect oligodendrocyte membrane currents (Chvátal et al., 1997, 1999a).

Tail currents in oligodendrocytes arise from the accumulation of K^+ in the ECS

Mature glial cells are almost exclusively permeable for K^+, thus depolarization of the glial membrane causes a massive efflux of K^+ out of the cell. Depolarization or hyperpolarization of oligodendrocytes in culture evokes currents that match the shape of the de- or hyperopolarizing pulse (Sontheimer and Kettenmann, 1988; Sontheimer et al., 1989). On the other hand, oligodendrocytes in corpus callosum or in spinal cord or oligodendrocyte-like cells in hippocampus are characterized by passive but decaying K^+ currents with prominent I_{tail} after the offset of the voltage jump (Fig. 4; Berger et al., 1991; Steinhäuser et al., 1992; Chvátal et al., 1995). Further analysis of oligodendrocyte currents revealed that in contrast to cultured cells where the ECS is almost infinite, current decays during voltage jumps and I_{tail}

observed in oligodendrocytes in brain slice preparations are produced by the extensive accumulation of K^+ in the vicinity of the oligodendrocyte membrane and by the shift of K^+ across the cell membrane either to or from the ECS. This conclusion is based on the following observations:

(1) Depolarizing or hyperpolarizing voltage jumps produce a more positive or negative shift of reversal potential (V_{rev}) of I_{tail}, respectively. This behavior is compatible with an efflux of K^+ during depolarization and an influx during hyperpolarization (Berger et al., 1991; Steinhäuser et al., 1992; Chvátal et al., 1997; Chvátal et al., 1999a).

(2) The time decay during, as well as after, the voltage jump is independent of voltage, but varies markedly from cell to cell. For any given cell the time constant of decay is voltage independent (Berger et al., 1991; Chvátal et al., 1999a).

(3) The glial membrane potential is strongly dependent on $[K^+]_e$. The superfusion of rat spinal cord slices with 55 mM K^+ shifts the reversal potential of all glial cell types, including oligodendrocytes, to –16 mV, which is

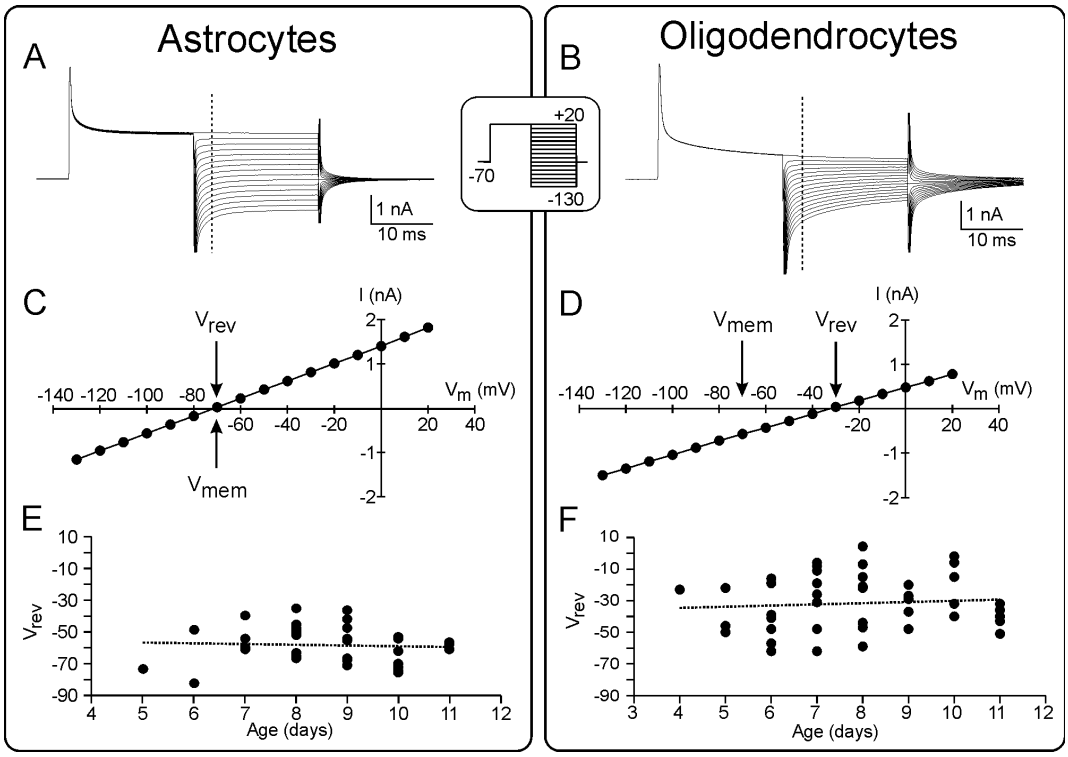


Fig. 4. Tail current analysis of the currents evoked in astrocytes and oligodendrocytes in the gray matter of spinal cord slices and the reversal potential of the tail currents during postnatal development. A, B: The membranes of astrocytes and oligodendrocytes were clamped from a holding potential of –70 mV to +20 mV for 20 ms. After this prepulse, the membrane was clamped for 20 ms to increasing de- and hyperpolarizing potentials (pattern of voltage commands in inset) ranging from –130 mV to +20 mV, at 10 mV increments. C, D: From traces as shown in A and B, currents (I) were measured five ms after the onset of the de- and hyperpolarizing pulses (dashed lines) and plotted as a function of the membrane potential (V_m). Glial membrane potentials (V_{mem}) and the reversal potentials (V_{rev}) after the depolarizing prepulse are indicated in the graphs by the arrows. In oligodendrocytes, in contrast to astrocytes, the depolarizing prepulse shifted the reversal potential from –70 mV to –31 mV. E, F: Reversal potential of the tail currents in astrocytes and oligodendrocytes during postnatal development. The values of reversal potential (V_{rev}) as a function of animal age and the corresponding linear regressions are shown for astrocytes and oligodendrocytes. Adapted from Chvátal et al., 1999.

close to the estimated equilibrium potential (–23 mV) as calculated from the Nernst equation (Chvátal et al., 1995).

(4) In mouse corpus callosum as well as in rat spinal cord gray matter, the application of Ba^{2+}, a K^+ channel blocker, inhibits I_{tail} after de- and hyperpolarizing voltage steps (Berger et al., 1991; Chvátal et al., 1995).

In summary, the I_{tail}, observed in oligodendrocytes, in white as well as in gray matter, do not arise from changes in the capacitance of the cell, but represent a rapid shift of K^+ caused by a change in the K^+ gradient across the cell membrane during the voltage step. Such a rapid shift of K^+ is mediated through K^+ channels or by K^+/Cl^- uptake (Kettenmann, 1986) but not by Na^+/K^+ ATPase activity, since the time-course of the tail currents is in the range of milliseconds, while the uptake of K^+ mediated via the activity of Na^+/K^+ ATPase may last several minutes (Kettenmann et al., 1987) and, in addition, requires an increased intracellular Na^+ concentration (Tang et al., 1980).

Oligodendrocyte tail currents reveal the inhomogeneity of the ECS

In experiments performed in the hippocampal slice or in the gray matter of the spinal cord slice, i.e. in tissue containing neurons as well as mature astrocytes and oligodendrocytes and their respective precursors (Steinhäuser et al., 1992; Chvátal et al., 1995), the occurrence of large I_{tail} was observed in oligodendrocytes or oligodendrocyte-like cells but not in other cell types (Fig. 4). Since the glial membrane is exclusively permeable for K^+, the Nernst equation $V_{rev} = (RT/F)\ \ln\ ([K^+]_e/[K^+]_i)$ can be used to calculate the extracellular K^+ concentration ($[K^+]_e$) from the values of V_{rev} of I_{tail} in the vicinity of the cell membrane. In oligodendrocyte-like cells in hippocampal slices, a +20 mV prepulse evokes a shift of V_{rev} of I_{tail} to –19 mV (Steinhäuser et al., 1992), which corresponds to 61 mM $[K^+]_e$. In the rat corpus callosum slice preparation, a +20 mV prepulse evokes in oligodendrocyte precursors an increase of $[K^+]_e$ up to 12 mM, while in mature oligodendrocytes it is 37 mM (Chvátal et al., 1997). In the rat spinal cord slice a +20 mV prepulse evokes in oligodendrocytes an increase of $[K^+]_e$ up to 47 mM, while in astrocytes it is 12 mM, in astrocyte precursors 15 mM and in oligodendrocyte precursors 22 mM (Chvátal et al., 1999a). These data indicate that the ECS is not homogeneous, i.e. it is more 'condensed' or 'compact' around mature oligodendrocytes than around precursor cells or mature astrocytes.

ECS inhomogeneities represented by the differences in K^+ accumulation around different cell types are also revealed by inducing cell swelling or shrinkage, i.e. changing the size of the ECS volume in spinal cord slices (Chvátal et al., 1999a, b). Cell swelling induced by the application of 50 mM K^+ or by hypotonic solution produces in the vicinity of astrocytes an increase in $[K^+]_e$ in the range of 200–240%, while in oligodendrocytes such an increase is only 22–30%, apparently because the ECS is already small around oligodendrocytes and cannot shrink below a certain level. Cell shrinkage evoked by hypertonic solutions has no effect on $[K^+]_e$ evoked by a depolarizing prepulse in astrocytes, while in oligodendrocytes a rapid decrease of $[K^+]_e$ is observed. These results also indicate that the swelling is more pronounced in astrocytes than in oligodendrocytes. It is possible to speculate that astrocytes are responsible for the majority of the cell volume changes in nervous tissue.

A model has been proposed that explains the differences in the K^+ shift across astrocytic and oligodendrocytic membranes on a cellular level (Fig. 5A; Chvátal et al., 1999a). The model is based on the assumption that during the depolarizing pulse, K^+ is extruded from the cell to the extracellular space. In astrocytes, extruded K^+ freely moves away from the membrane, and within several milliseconds a new steady-state is established, i.e. the same amount of K^+ that is leaving the cell is freely redistributed in the ECS. After the offset of the voltage command, only a small amount of K^+ re-enters the cell, creating a very small I_{tail}. In oligodendrocytes, presumed diffusion barriers (Fig. 5A, dashed ellipse) prevents K^+ from moving away freely and thus K^+ accumulates in the vicinity of the cell membrane. During this accumulation, a new K^+ equilibrium is established, which is reflected by the decreasing current during the voltage step. After the offset of the depolarizing prepulse, K^+ moves back into the cell and produces

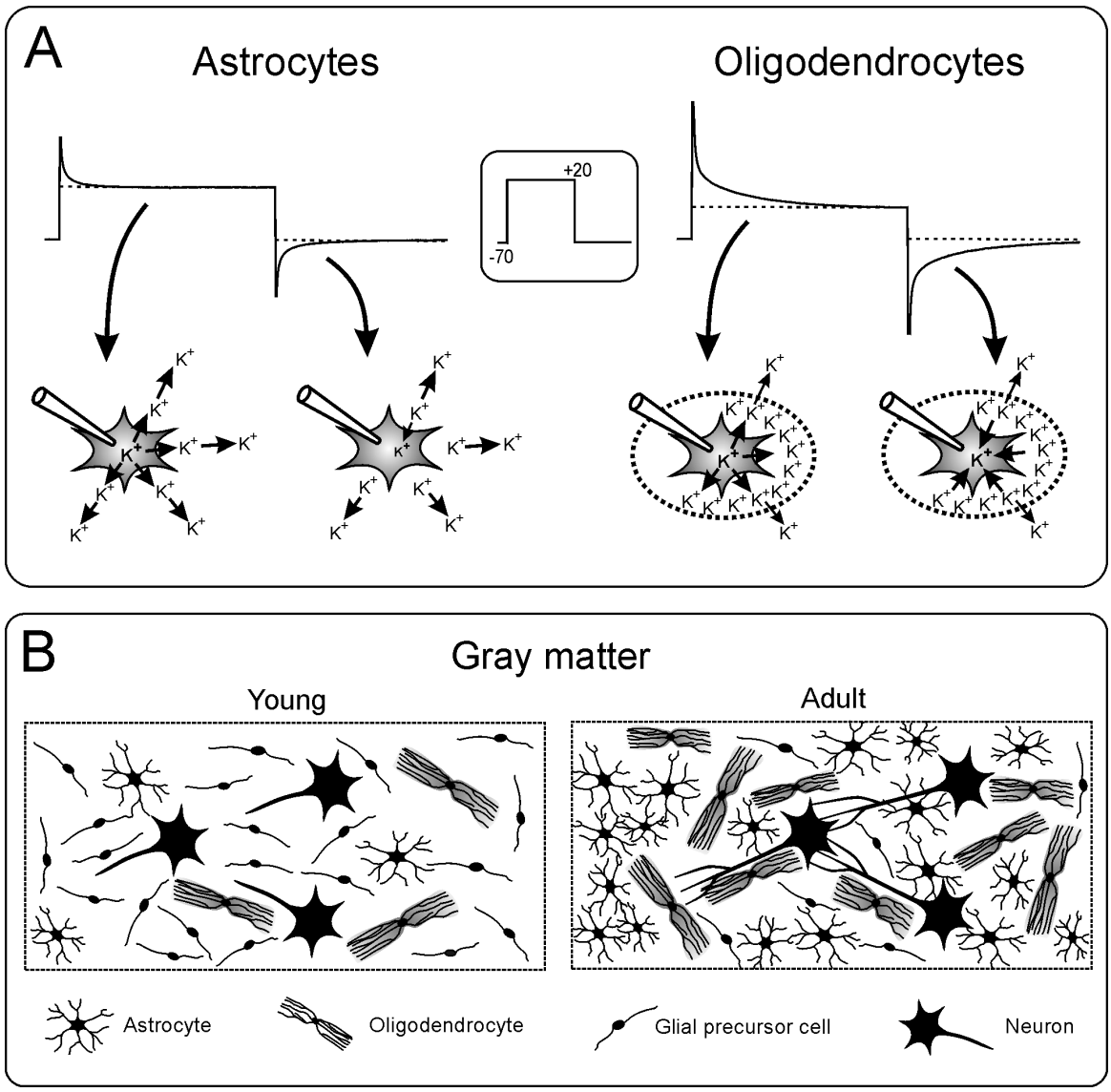


Fig. 5. A model of K^+ movements across the astrocytic and oligodendrocytic membrane (A) and a hypotheses of the developmental changes in the spinal cord gray matter (B) A: Hypothesis of the K^+ shift across astrocytic and oligodendrocytic membranes during and after the depolarizing pulse. In astrocytes, a small amount of K^+ re-enters the cell after the offset of the voltage command, creating a very small tail current. In oligodendrocytes, K^+ accumulates in the vicinity of the cell membrane due to presumed diffusion barriers (dashed ellipse) and thus produces prominent tail currents after the offset of the voltage command. B: Drawing of the developmental changes in the spinal cord gray matter. In the nervous tissue of young animals the majority of the cells are glial precursors and the ECS volume remains relatively large. In adulthood there is a substantial increase in the number of mature astrocytes and oligodendrocytes together with the elongation and branching of glial processes, axonal and dendritic outgrowth and a decrease of the ECS volume. The gray shading between oligodendrocyte processes indicates clusters of the 'compact' ECS in the vicinity of the oligodendrocyte membrane. Adapted from Chvátal et al., 1999.

prominent I_{tail}. The time-course of the K^+ shifts is in the range of milliseconds, and thus they are likely not mediated by ATP-based uptake mechanisms.

Tail currents and ECS volume during the maturation of the CNS

The possible link between I_{tail}, i.e. large and fast transmembrane shifts of K^+, and the ECS size was also suggested in experiments in which the membrane properties of cells of the oligodendrocyte lineage were compared with the ECS diffusion parameters of rat corpus callosum during development, at the period of extensive myelination (Chvátal et al., 1997). The real-time iontophoretic method was used to determine the absolute values of ECS diffusion parameters and their dynamic changes in nervous tissue in situ (Syková, 1997; Nicholson and Syková, 1998). At P10, when the majority of cells in the rat brain white matter are glial precursors, no I_{tail} were observed in these cells and the ECS volume fraction was $\alpha = 0.36$, i.e. the size of the ECS was 36%. In contrast, in white matter after myelination at P20, characterized by the presence of mature oligodendrocytes with decaying currents and large I_{tail} after the offset of a voltage jump, the ECS volume fraction was significantly smaller, $\alpha = 0.25$. These studies demonstrate that the appearance of I_{tail} in oligodendrocytes during early postnatal development coincides with the decrease of the ECS volume (Chvátal et al., 1997).

The above studies raise the question whether the appearance and increase of I_{tail} in oligodendrocytes develop concomitantly with the 'maturation' of the nervous tissue, i.e. the outgrowth and prolongation of cell processes and changes in the extracellular matrix composition, or whether the presence of large I_{tail} in individual cells is independent of the overall decrease of the ECS volume during early postnatal development. It was shown in the study of Prokopová et al. (1997) that the extracellular volume fraction, measured over a volume on the order of 10^{-3} mm^3, significantly decreases in spinal cord gray matter during postnatal development between P4–5 and P7–8. On the other hand, values of V_{rev} of I_{tail} in patch-clamp experiments, which represent the changes in $[K^+]_e$ not in the bulk of the tissue but in the immediate vicinity of the glial membrane, do not significantly change among all glial cell types studied between P5 and P11 (Fig. 4). The appearance of I_{tail} in oligodendrocytes seems not to be related to the average ECS volume fraction of the brain tissue but rather to the increasing number of mature oligodendrocytes surrounded by the 'compact' ECS (Fig. 5B). Such an increased number of mature oligodendrocytes during postnatal development has been found in spinal cord gray matter (Chvátal et al., 1995). An increased number of oligodendrocytes surrounded by a 'compact' ECS might be, together with the elongation and branching of glial processes (Takahashi et al., 1990) and axonal and dendritic outgrowth (Bicknell and Beal, 1984), an additional factor responsible for the decrease in the overall ECS volume during postnatal development (Chvátal et al., 1999a).

The questions remain, do inhomogenities of the ECS affect neuronal signaling, what are the obstacles or diffusion barriers which are present around oligodendrocytes and prevent K^+ from moving away from the oligodendrocyte membrane, and how might these barriers affect neuronal function? The finding that tail currents in oligodendrocytes are observed in brain slices but not in tissue culture with an infinite ECS volume (Sontheimer and Kettenmann, 1988; Sontheimer et al., 1989), indicates that the diffusion barriers arise, at least partially, from the compartments of the nervous tissue. The larger accumulation of K^+ around oligodendrocytes is most likely caused by a smaller ECS around this type of cell, but regional differences in molecules of the extracellular matrix, which have been shown to be produced by glial cells, cannot be excluded (Maleski and Hockfield, 1997; for review see Celio et al., 1998). The unique electrophysiological behavior of oligodendrocytes could also be correlated with their specific morphology, i.e. long and parallel processes, not seen in other types of glial cells, which probably corresponds to their tight contact with axons.

Patch-clamp studies of the electrophysiological properties of glial cells in different regions of the CNS and in different species have revealed that mature oligodendrocytes are surrounded by clusters of compact ECS. Since the number of oligoden-

drocytes increases during early postnatal development, such clusters in the CNS may contribute to the overall decrease of the ECS volume fraction in the brain.

Role of glial cells in ionic homeostasis and in neuronal signaling

It is evident that during development of the ECS, the membrane properties of glial cells change concomitantly with the maturation of the nervous tissue, i.e. axonal outgrowth, branching of the neuronal processes and formation of the synapses. During the first 2–3 weeks after birth the cellular composition of the nervous tissue changes: the number of glial precursor cells decreases and the number of mature astrocytes and oligodendrocytes increases (Chvátal et al., 1995). It was shown that the ability of mature nervous tissue to efficiently buffer K^+ and pH changes is closely related to gliogenesis (Jendelová and Syková, 1991) and that impairment of glial cell maturation by early postnatal X-irradiation alters K^+ and pH homeostasis (Syková et al., 1992; Syková and Chvátal, 1993) and blocks the normal pattern of volume fraction decrease during postnatal development, resulting in a significant increase in the ECS volume (Syková et al., 1996). Glial cells do not form synapses, however they interact with neurons and with other distant cells by the diffusion of ions and other neuroactive substances through the volume of ECS, i.e. by 'volume transmission' (Agnati et al., 1995; Syková, 1997; Nicholson and Syková, 1998; Zoli et al., 1999). The presence of voltage- and ligand-activated channels on glial precursor cells and on mature astrocytes and oligodendrocytes are very important prerequisites for the volume transmission and are involved in the maintenance of ionic homeostasis and brain energy metabolism, neuron-to-glia and glia-to-neuron signaling, synaptic transmission and neuronal function, and the changes of ECS volume.

Maintenance of ionic homeostasis

It was already shown in the study of Orkand et al. (1966) that during neuronal activity, K^+ increases in the relatively small ECS. It was therefore proposed that to prevent extensive accumulation of K^+, which may impair neuronal signaling, glial cells regulate extracellular K^+ concentration by spatial K^+ buffering. Since the glial membrane is permeable to K^+ and glial cells create a syncitium, this buffering mechanism is maintained by the K^+ influx into the glial cell in the region of increased K^+, which is then released in the distant regions of the CNS. Some studies indicate that K_{IR} are also important for spatial buffer currents (Sontheimer, 1994). In addition to spatial K^+ buffering, K^+ is removed from the ECS by Na^+, K^+-ATPase. It has been suggested in a Na^+ recycling model that astrocyte Na^+ channels are involved in extracellular K^+ homeostasis by providing a 'return pathway' for Na^+ that is required for Na^+, K^+-ATPase operation during neuronal firing (Sontheimer et al., 1993; Sontheimer, 1994). Glial cells may be also involved in the maintenance of Cl^- homeostasis during neuronal activity (Fig. 6B; Bormann and Kettenmann, 1988). The release of GABA from presynaptic nerve terminals results in an influx of Cl^- from the ECS into the postsynaptic neuron and $[Cl^-]_e$ decreases. GABA receptors on glial cells that are activated in the vicinity of the synaptic cleft could cause an efflux of Cl^- and K^+ from glial cells and could be operative in the rapid restoration of $[Cl^-]_e$.

Glial cells also play an important role in buffering activity-related alkaline changes in extracellular pH (Chesler, 1990; Syková, 1992; Deitmer and Rose, 1996). Some of the membrane transport processes regulating intra- and extracellular pH, such as Na^+/H^+ exchange and $Na^+/H^+/Cl^-/HCO_3^-$ cotransport, are present in both neurons and glia, while others are specific either for neurons (e.g. H^+ channels, H^+ or HCO_3^- permeability of the ionic channels activated by GABA or glutamate) or for glia (e.g. voltage-dependent Na^+-HCO_3^- cotransport and lactate extrusion). The glial cell membrane is also readily permeable to CO_2, which reacts with H_2O to form carbonic acid, which in turn quickly dissociates into water and protons. This reaction is enhanced by the catalytic action of the enzyme carbonic anhydrase, which is almost exclusively present in glial cells.

Brain energy metabolism

Recent studies indicate that neurotransmitters such as noradrenaline, vasoactive intestinal peptide,

adenosine or ATP may regulate the glucose fluxes and glycogen metabolism in astrocytes (Pellerin et al., 1997, for reviews see Magistretti and Pellerin, 1996; Tsacopoulos and Magistretti, 1996; Magistretti et al., 1999; see also Chapter 11 by Magistretti and Pellerin). Glutamate released from neurons is taken up by astrocytes, where it is converted into glutamine. Glutamine is released by astrocytes and taken up by neuronal terminals, where it is converted back to glutamate to re-establish its physiological levels (for review see Westergaard et al., 1995). Glutamine delivery to neurons is maintained by the volume transmission through the ECS. In addition, glutamate released from neurons stimulates aerobic glycolysis in astrocytes and thus provides lactate to neurons to fulfill their energy needs. This process is activated in several steps: glutamate uptake via Na^+-dependent transporters, activation of Na^+, K^+-ATPase, activation of glucose uptake, lactate release and finally, utilization of lactate by neurons. It was shown that neurotransmitters stimulate glucose utilization by the enhanced formation of glycogen, the major energy reserve in the brain.

Neuron-to-glia and glia-to-neuron signaling

During neuronal activity neurons release not only neurotransmitters, which evoke responses in postsynaptic cells and in glial cells located in the

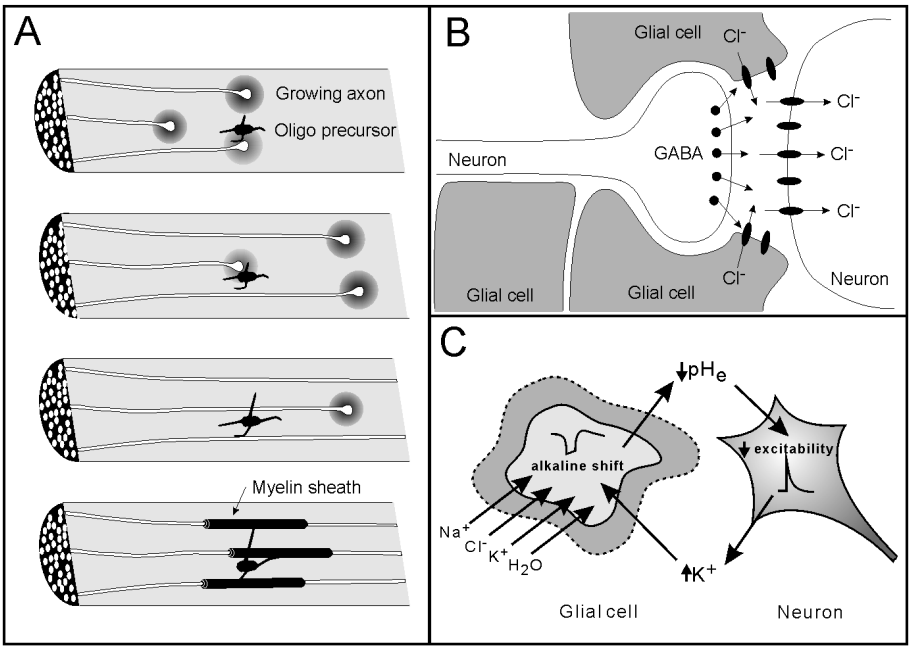


Fig. 6. Some of the proposed mechanisms of neuron-to-glia and glia-to-neuron interactions. A: Oligodendrocyte precursors may detect neurotransmitters released from the growth cones of migrating neurites. Neurotransmitter release thus may initiate oligodendrocyte maturation and start the ensheathment of the axons (adapted from Kettenmann et al., 1992). B: Maintenance of Cl^- homeostasis by glial cells during neuronal activity. The release of GABA into the synaptic cleft results in an influx of Cl^- into the postsynaptic neuron and $[Cl^-]_e$ decreases. Activation of GABA receptors on glial cells may in turn cause an efflux of Cl^- from glial cells and thus restore $[Cl^-]_e$ (adapted from Bormann and Kettenmann, 1988). C: A model of a non-specific feedback mechanism suppressing neuronal activity. Firing neurons release K^+, which accumulates in the extracellular space and thus depolarizes glial cells. This results in an increase of glial intracellular pH, a decrease of extracellular pH and the suppression of neuronal activity. Ionic transmembrane shifts also result in cell swelling, a decrease of the ECS volume and the larger accumulation of ions and neuroactive substances in the ECS (adapted from Syková, 1997).

vicinity of the synapse, but they also release a variety of other neuroactive substances, e.g. ions, transmitters, neurohormones, peptides and metabolites, which diffuse via the ECS to adjacent neurons and glial cells. The effect of such a release may be either short-term, e.g. ion regulation, release of substrates, transmitter clearance, or long-term, e.g. mitosis, regeneration, neuronal guidance during development or the onset of myelination, and is often associated with an increase of intracellular Ca^{2+} (for review see Verkhratsky et al., 1998). It has been proposed, for example, that the release of K^+ or other neuroactive substances, e.g. GABA, from neurons may regulate glial cell differentiation and myelin formation in white matter (Fig. 6A; Kettenmann et al., 1992). Oligodendrocyte precursors may detect the releasing transmitter from the growth cones of the migrating neurites and start the ensheathment of the axons. When the migration ends, the release of transmitter stops and thus the development and maturation of oligodendrocytes may halt.

Glial cells, in response to excitatory amino acid receptor activation, may also release growth factors, neuropeptides or neurotransmitters during development as well as during adulthood (for review see Gallo and Russell, 1995). For example, in astrocyte-neurone co-cultures bradykinin significantly increased calcium levels in neurons co-cultured with astrocytes, but not in single neurons (Parpura et al., 1994). The bradykinin-induced neuronal calcium elevation was blocked by the glutamate receptor antagonists D-2-amino-5-phosphonopentaonic acid or D-glutamylglycine. It was therefore suggested that astrocytes regulate neuronal calcium levels through the Ca^{2+}-dependent release of glutamate. If such a release of neuroactive substances, which are known to affect neuronal development, also occurs in vivo, glial cells may participate in activity-dependent plasticity in the adult brain (Gallo and Russell, 1995).

Synaptic transmission and neuronal function

It was shown in a number of studies that in the mature CNS, glial cell processes encapsulate synapses, thus several functions have been suggested for glial cells at the synaptic site (for reviews see Barres, 1991; Porter and Mccarthy, 1997). The glial cells associated with synapses are thought to take up transmitters, such as glutamate, to terminate neurotransmitter action; they may provide synaptic insulation to prevent neurotransmitter spillover to nearby synapses; they may accumulate Ca^{2+} from the synaptic cleft and reduce Ca^{2+}-dependent transmitter release; and they may even participate in long-term potentiation by synthesizing and releasing arachidonic acid, which induces a long-term, activity-dependent enhancement of synaptic transmission in the hippocampus.

Ritchie and colleagues have proposed in a Na^+ channel transfer model that neuroglial cells may support neuronal function by donating Na^+ channels to nearby axons to reduce the biosynthetic load of neurons, since the half-life of Na^+ channels has been estimated to be about 2 days (Bevan et al., 1985; Ritchie, 1988; for review see Oh, 1997). There is no compelling evidence that this transference exists in the nervous tissue, but at least three observations may support it: Na^+ channels are localized at the node of Ranvier as well as in astrocytes in the perinodal regions; the same Na^+ channel genes are expressed in neurons and glial cells; and the translocation of large protein molecules from axons to the adjacent glial cells has been observed.

Changes of ECS volume

Glial cell swelling is an accompanying phenomenon of normal and many pathological states; a link between ionic and volume changes in glial cells and signal transmission has been proposed in a model of a non-specific feedback mechanism suppressing neuronal activity (Fig. 6C; Syková, 1997). First, neuronal activity results in the accumulation of $[K^+]_e$, which in turn depolarizes glial cells, and this depolarization induces an alkaline shift in glial pH_i. Second, the glial cells extrude acid and the resulting acid shift causes a decrease in neuronal excitability. The ionic transmembrane shifts are accompanied by water movement, and thus this feedback mechanism may be amplified by activity-related glial swelling compensated for by ECS volume shrinkage and by increased tortuosity, presumably by the crowding of molecules of the

ECS matrix and/or by the swelling of fine glial processes. This, in turn, results in a larger accumulation of ions and other neuroactive substances in the nervous tissue due to more hindered diffusion in the ECS and thus affects volume transmission (Syková, 1997). In addition to $[K^+]_e$ increase, the activation of different neurotransmitter receptors on glial cells may also change astroglial morphology and volume and affect the excitability of neurons (for review see Porter and McCarthy, 1997). Electrically induced seizures in the hippocampal slice, for example, can be prevented by furosemide, which is known to block astrocytic swelling (Hochman et al., 1995; Syková et al., 1999). Such receptor-mediated communication between neurons and astrocytes may provide a feedback of the astrocytic regulation of neuronal excitability (Porter and McCarthy, 1997). In addition, clusters of compact ECS around oligodendrocytes revealed by tail current analysis in the corpus callosum as well as in the gray matter of the spinal cord (Chvátal et al., 1997, 1999a), may facilitate spatial K^+ buffering and selectively affect the diffusion of neuroactive substances in the CNS, i.e. volume transmission, in specific areas and/or directions.

Concluding remarks

Studies of glial cells during a period of more than 100 years have shown that they have distinct electrophysiological and morphological properties and that they play an important role in different brain functions, a role that was for many years under-estimated. Astrocytes and oligodendrocytes in the ECS express voltage- as well as ligand-activated currents that are involved in the maintainance of ionic and volume homeostasis as well as neuronal functions, in the release of neuroactive substances, in energy metabolism, in synaptic transmission and in volume transmission (see also Chapter 6 by Syková et al.). However, to fully understand how glial cells participate in neuronal signaling, recent studies of glial cells have focused on their role during pathological states such as anoxia and ischemia, during neurodegenerative diseases, in brain tumors and during aging and regeneration of the nervous tissue. Such research should help to elucidate the complex relationship between neurons and glial cells in normal as well as pathologically altered nervous tissue and to establish new transplantation and regeneration procedures in the treatment of CNS diseases.

Acknowledgements

Supported by grants VS 96 130, GAČR 307/96/K226, GAČR 309/97/K048, GAČR 309/99/0657 and GAČR 305/99/0655.

List of abbreviations

AMPA	a-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ATP	adenosinetriphosphate
CNS	central nervous system
CNQX	6-cyano-7-nitroqinoxaline-2,3-dione
ECS	extracellular space
GABA	γ-aminobutyric acid
GFAP	glial fibrillar acidic protein
I_{tail}	tail current
$[K^+]_e$	extracellular concentration of potassium ions
K_{DR}	potassium delayed outwardly rectifying currents
K_A	A-type potassium current
K_{IR}	potassium inwardly rectifying currents
NMDA	N-methyl-D-aspartate
P	postnatal day
PCR	polymerase chain reaction
V_{rev}	reversal potential
V_{mem}	membrane potential

References

Agnati, L.F., Zoli, M., Strömberg, I. and Fuxe, K. (1995) Intercellular communication in the brain: wiring vs. volume transmission. *Neuroscience*, 69: 711–726.

Akopian, G., Kressin, K., Derouiche, A. and Steinhäuser, C. (1996) Identified glial cells in the early postnatal mouse hippocampus display different types of Ca^{2+} currents. *Glia*, 17: 181–194.

Anděrová, M., Chvátal, A., Eliasson, C., Pěkný, M., Syková, E. (1999) Membrane properties and swelling of astrocytes in GFAP–/– mice. *Physiol. Res.*, Suppl. 1., 48: 52.

Backus, K.H. and Berger, T. (1995) Developmental variation of the permeability to Ca^{2+} of AMPA receptors in presumed hilar glial precursor cells. *Pflügers Arch.*, 431: 244–252.

Barres, B.A. (1991) New roles for glia. *J. Neurosci.*, 11: 3685–3694.

Barres, B.A., Koroshetz, W.J., Chun, L.L. Y. and Corey, D.P. (1990) Ion channel expression by white matter glia: the type–1 astrocyte. *Neuron.*, 5: 527–544.

Berger, T. (1995) AMPA-type glutamate receptors in glial precursor cells of the rat corpus callosum: ionic and pharmacological properties. *Glia*, 14: 101–114.

Berger, T., Schnitzer, J. and Kettenmann, H. (1991) Developmental changes in the membrane current pattern, K^+ buffer capacity, and morphology of glial cells in the corpus callosum slice. *J. Neurosci.*, 11: 3008–3024.

Berger, T., Walz, W., Schnitzer, J. and Kettenmann, H. (1992) GABA- and glutamate-activated currents in glial cells of the mouse corpus callosum slice. *J. Neurosci. Res.*, 31: 21–27.

Berger, T., Müller, T. and Kettenmann, H. (1995) Developmental regulation of ion channels and receptors on glial cells. *Persp. Dev. Neurobiol.*, 2: 347–356.

Bernstein, M., Lyons, S.A., Möller, T. and Kettenmann, H. (1996) Receptor-mediated calcium signalling in glial cells from mouse corpus callosum slices. *J. Neurosci. Res.*, 15: 152–163.

Bevan, S., Chiu, S.Y., Gray, P.T.A. and Ritchie, J.M. (1985) The presence of voltage-gated sodium, potassium and chloride channels in rat cultured astrocytes. *Proc. R. Soc. (Lond.)*, 225: 229–313.

Bicknell, H.R. and Beal, J.A. (1984) Axonal and dendritic development of substantia gelatinosa neurons in the lumbosacral spinal cord of the rat. *J. Comp. Neurol.*, 226: 508–522.

Bordey, A. and Sontheimer, H. (1997) Postnatal development of ionic currents in rat hippocampal astrocytes in situ. *J. Neurophysiol.*, 78: 461–477.

Bormann, J. and Kettenmann, H. (1988) Patch-clamp study of γ-aminobutyric acid receptor Cl^- channels in cultured astrocytes. *Proc. Natl Acad. Sci. USA.*, 85: 9336–9340.

Brockhaus, J., Illschner, S., Banati, R.B. and Kettenmann, H. (1993) Membrane properties of ameboid microglial cells in the corpus callosum slice from early postnatal mice. *J. Neurosci.*, 13: 4412–4421.

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

Chesler, M. (1990) The regulation and modulation of pH in the nervous system. *Prog. Neurobiol.*, 34: 401–427.

Chvátal, A., Pastor, A., Mauch, M., Syková, E. and Kettenmann, H. (1995) Distinct populations of identified glial cells in the developing rat spinal cord slice: ion channel properties and cell morphology. *Eur.J. Neurosci.*, 7: 129–142.

Chvátal, A., Berger, T., Vořišek, I., Orkand, R.K., Kettenmann, H. and 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., Anděrová, M., Žiak, D. and Syková, E. (1999a) 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.

Chvátal, A., Anděrová, M., Vargová, L., Prokopová, Š., Žiak, D. and Syková, E. (1999b) Effect of osmotic stress on glial potassium tail currents and extracellular space diffusion parameters in the rat spinal cord slice. *Physiol. Res.*, Suppl. 1., 48: 61.

Deitmer, J.W. and Rose, C.R. (1996) pH regulation and proton signaling by glial cells. *Prog. Neurobiol.*, 48: 73–103.

Eder, C. (1998) Ion channels in microglia (brain macrophages). *Am. J. Physiol.*, 275: C327-C342.

Edwards, F.A., Konnerth, A., Sakmann, B. and Takahashi, T. (1989) A thin slice preparation for patch clamp recordings from neurones of the mammalian central nervous system. *Pflügers Arch.*, 414: 600–612.

Gallo, V. and Russell, J.T. (1995) Excitatory amino acid receptors in glia: different subtypes for distinct function? *J. Neurosci. Res.*, 42: 1–8.

Grosche, J., Matyash, V., Möller, T., Verkhratsky, A., Reichenbach, A. and Kettenmann, H. (1999) Microdomains for neuron-glia interaction: parallel fiber signalling to Bergmann glial cells. *Nature Neurosci.*, 2: 139–143.

Hild, W. and Tasaki, I. (1962) Morphological and physiological properties of neurons and glial cells in tissue culture. *J. Neurophysiol.*, 25: 277–304.

Hochman, D.W., Baraban, S.C. Owens, J.W.M. and Schwartzkroin, P.A. (1995) Dissociation of synchronization and excitability in furosemide blockade of epileptiform activity. *Science*, 270: 99–102.

Jabs, R., Kirchhoff, F., Kettenmann, H. and Steinhäuser, C. (1994) Kainate activates Ca^{2+}-permeable glutamate receptors and blocks voltage-gated K^+ currents in glial cells of mouse hippocampal slices. *Pflügers Arch.*, 426: 310–319.

Jendelová, P. and Syková, E. (1991) Role of glia in K^+ and pH homeostasis in the neonatal rat spinal cord. *Glia*, 4: 56–63.

Kettenmann, H. (1986) Oligodendrocytes control extracellular potassium by active uptake and spatial buffering. *Adv. Biosci.*, 61: 155–163.

Kettenmann, H., Syková, E., Orkand, R.K. and Schachner, M. (1987) Glial potassium uptake following depletion by intracellular ionophoresis. *Pflügers Arch.*, 410: 1–6.

Kettenmann, H., Blankenfeld, G.V. and Trotter, J. (1992) Physiological properties of oligodendrocytes during development. *Ann. NY Acad. Sci. USA*, 633: 64–77.

Kirchhoff, F., Mülhardt, C., Pastor, A., Becker, C.-M. and Kettenmann, H. (1996) Expression of glycine receptor subunits in glial cells of the rat spinal cord. *J. Neurochem.*, 66: 1383–1390.

Kirischuk, S., Möller, T., Voitenko, N., Kettenmann, H. and Verkhratsky, A. (1995) ATP-induced cytoplasmatic calcium mobilisation in Bergmann glial cells. *J. Neurosci.*, 15: 7861–7871.

Kirischuk, S., Tuschick, S., Verkhratsky, A. and Kettenmann, H. (1996) Calcium signalling in mouse Bergmann glial cells mediated by α_1-adrenoreceptors and H_1 histamine receptors. *Eur. J. Neurosci.*, 8: 1198–1208.

Kressin, K., Kuprijanova, E., Jabs, R., Seifert, G. and Steinhäuser, C. (1995) Developmental regulation of Na^+ and K^+ conductances in glial cells of mouse hippocampal brain slices. *Gli*, 15: 173–187.

Kuffler, S.W., Nicholls, J.G. and Orkand, R.K. (1966) Physiological properties of glial cells in the central nervous system of amphibia. *J. Neurophysiol.*, 29: 768–787.

Magistretti, P.J. and Pellerin, L. (1996) Cellular bases of brain energy metabolism and their relevance to functional brain imaging: Evidence for a prominent role of astrocytes. *Cereb. Cort.*, 6: 50–61.

Magistretti, P.J., Pellerin, L., Rothman, D.L. and Shulman, R.G. (1999) Energy on demand. *Science*, 283: 496–497.

Maleski, M. and Hockfield, S. (1997) Glial cells assemble hyaluronan-based pericellular matrices in vitro. *Glia*, 20: 193–202.

Müller, T., Möller, T., Berger, T., Schnitzer, J. and Kettenmann, H. (1992) Calcium entry through kainate receptors and resulting potassium-channel blockade in Bergmann glial cells. *Science*, 256: 1563–1566.

Müller, T., Grosche, J., Ohlemeyer, C. and Kettenmann, H. (1993) NMDA-activated currents in Bergmann glial cells. *NeuroReport*, 4: 671–674.

Müller, T., Fritschy, J.M., Grosche, J., Pratt, G.D., Mohler, H. and Kettenmann, H. (1994) Developmental regulation of voltage-gated K^+ channel and $GABA_A$ receptor expression in Bergmann glial cells. *J. Neurosci.*, 14: 2503–2514.

Müller, T., Möller, T., Neuhaus, J. and Kettenmann, H. (1996) Electrical coupling among Bergmann glial cells and its modulation by glutamate receptor activation. *Glia*, 17: 274–284.

Nicholson, C. and Syková, E. (1998) Extracellular space structure revealed by diffusion analysis. *Trends Neurosci.*, 21: 207–215.

Oh, Y. (1997) Ion channels in neuroglial cells. *Kaohsiung. J. Med. Sci.*, 13: 1–9.

Orkand, R.K., Nicholls, J.G. and Kuffler, S.W. (1966) Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. *J. Neurophysiol.*, 29: 788–806.

Parpura, V., Basarsky, T.A., Liu, F., Jeftinija, K., Jeftinia, S. and Haydon, P. (1994) Glutamate-mediated astrocyte-neuron signaling. *Nature*, 369: 744–747.

Pastor, A., Chvátal, A., Syková, E. and Kettenmann, H. (1995) Glycine- and GABA-activated currents in identified glial cells of the developing rat spinal cord slice. *Eur. J. Neurosci.*, 7: 1188–1198.

Pellerin, L., Stolz, M., Sorg, O., Martin, J.-L., Deschepper, C.F. and Magistretti, P.J. (1997) Regulation of energy metabolism by neurotransmitters in astrocytes in primary culture and in an immortalized cell line. *Glia*, 21: 74–83.

Porter, J.T. and McCarthy, K.D. (1995) GFAP-positive hippocampal astrocytes in situ respond to glutamatergic neuroligands with increases in $[Ca^{2+}]_i$. *Glia*, 13: 101–112.

Porter, J.T. and McCarthy, K.D. (1996) Hippocampal astrocytes in situ respond to glutamate released from synaptic terminals. *J. Neurosci.*, 16: 5073–5081.

Porter, J.T. and McCarthy, K.D. (1997) Astrocytic neurotransmitter receptors in situ and in vivo. *Prog. Neurobiol.*, 51: 439–455.

Prokopová, Š., Vargová, J. and Syková, E. (1997) Heterogenous and anisotropic diffusion in the developing rat spinal cord. *NeuroReport*, 8: 3527–3532.

Ritchie, J.M. (1988) Sodium-channel turnover in rabbit cultured Schwann cells. *Proc. R. Soc. (Lond.)*, 223: 423–430.

Seifert, G. and Steinhäuser, C. (1995) Glial cells in the mouse hippocampus express AMPA receptors with an intermediate Ca^{2+} permeability. *Eur. J. Neurosci.*, 7: 1872–1881.

Sontheimer, H. (1994) Voltage-dependent ion channels in glial cells. *Glia*, 11: 156–172.

Sontheimer, H. and Kettenmann, H. (1988) Heterogeneity of potassium currents in cultured oligodendrocytes. *Glia*, 1: 415–420.

Sontheimer, H. and Waxman, S.G. (1993) Expression of voltage-activated ion channels by astrocytes and oligodendrocytes in the hippocampal slice. *J. Neurophysiol.*, 70: 1863–1873.

Sontheimer, H., Trotter, J., Schachner, M. and Kettenmann, H. (1989) Channel expression correlates with differentiation stage during the development of oligodendrocytes from their precursor cells in culture. *Neuron*, 2: 1135–1145.

Sontheimer, H., Fernandez-Marques, E., Ullrich, N., Pappas, C.A. and Waxman, S.G. (1993) Astrocyte Na^+ channels are required for maintenance of Na^+/K^+-ATPase activity. *J. Neurosci.*, 14: 2464–2475.

Steinhäuser, C. and Gallo, V. (1996) News on glutamate receptors in glial cells. *Trends Neurosci.*, 19: 339–345.

Steinhäuser, C., Berger, T., Frotscher, M. and Kettenmann, H. (1992) Heterogeneity in the membrane current pattern of identified glial cells in the hippocampal slice. *Eur.J. Neurosci.*, 4: 472–484.

Steinhäuser, C., Jabs, R. and Kettenmann, H. (1994) Properties of GABA and glutamate responses in identified glial cells of the mouse hippocampal slice. *Hippocampus*, 4: 19–36.

Sturrock, R.R. (1980) Myelination of the mouse corpus callosum. *Neuropathol. Appl. Neurobiol.*, 6: 415–420.

Syková, E. (1992) Ionic and volume changes in the microenvironment of nerve and receptor cells. In: D. Ottoson, (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. and Chvátal, A. (1993) Extracellular ionic and volume changes: the role in glia-neuron interaction. *J. Chem. Neuroanatom.*, 6: 247–260.

Syková, E., Jendelová, P., Šimonová, Z. and Chvátal, A. (1992) K^+ and pH homeostasis in the developing rat spinal cord is impaired by early postnatal X-irradiation. *Brain Res.*, 594: 19–30.

Syková, E., Svoboda, J., Šimonová, Z., Lehmenkühler, A. and Lassmann, H., (1996) X-irradiation-induced changes in the diffusion parameters of the developing rat brain. *Neuroscience*, 70: 597–612.

Syková, E., Vargová, L., Prokopová, Š. and Šimonová, Z. (1999) Glial swelling and astrogliosis produce diffusion barriers in the rat spinal cord. *Glia*, 25: 56–70.

Takahashi, T., Misson, J.-P. and Caviness, V.S., Jr. (1990) Glial process elongation and branching in the developing murine

neocortex: a qualitative and quantitative immunohistochemical analysis. *J. Comp. Neurol.*, 302: 15–28.

Tang, C.-M., Cohen, M.W. and Orkand, R.K. (1980) Electrogenic pumps in axons and neuroglia and extracellular potassium homeostasis. *Brain Res.*, 194: 283–286.

Tsacopoulos, M. and Magistretti, P.J. (1996) Metabolic coupling between glia and neurons. *J. Neurosci.*, 16: 877–885.

Tse, F.W., Fraser, D.D., Duffy, S. and MacVicar, B.A. (1992) Voltage-activated K^+ currents in acutely isolated hippocampal astrocytes. *J. Neurosci.*, 12: 1781–1788.

Verkhratsky, A., Orkand, R.K. and Kettenmann, H. (1998) Glial calcium: Homeostasis and signaling function. *Physiol. Rev.*, 78: 99–141.

Walz, W. (1989) Role of glial cells in the regulation of the brain ion microenvironment. *Prog. Neurobiol.*, 33: 309–333.

Westergaard, N., Sonnewald, U. and Schousboe, A. (1995) Metabolic trafficking between neurons and astrocytes: the glutamate/glutamine cycle revisited. *Dev. Neurosci.*, 17: 203–211.

Žiak, D., Chvátal, A. and Syková, E. (1998) Glutamate-, kainate- and NMDA-evoked membrane currants in identified glial cells in rat spinal cord slice. *Physiol. Res.*, 47: 365–375.

Zoli, M., Jansson, A., Syková, E., Agnati, L.F. and Fuxe, K. (1999) Volume transmission in the CNS and its relevance for neuropsychopharmacology. *TIPS*, 20: 142–150.