Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 90 (2008) 135 – 147
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Review

Microdialysis of GABA and glutamate: Analysis, interpretation and comparison with microsensors

Miranda van der Zeyden [a,*], Weite H. Oldenziel [b], Kieran Rea [c], Thomas I. Cremers [a,b], Ben H. Westerink [a,b]

[a] *Department of Biomonitoring and Sensoring, University Centre for Pharmacy, Antonius Deusinglaan 1, Groningen, The Netherlands*
[b] *Brains-on-Line, Antonius Deusinglaan 1, Groningen, The Netherlands*
[c] *Department of Pharmacology and Therapeutics, National University of Ireland, Galway, Ireland*

Available online 14 September 2007

Abstract

GABA and glutamate sampled from the brain by microdialysis do not always fulfill the classic criteria for exocytotic release. In this regard the origin (neuronal *vs.* astroglial, synaptic *vs.* extrasynaptic) of glutamate and GABA collected by microdialysis as well as in the ECF itself, is still a matter of debate. In this overview microdialysis of GABA and glutamate and the use of microsensors to detect extracellular glutamate are compared and discussed.

During basal conditions glutamate in microdialysates is mainly derived from non-synaptic sources. Indeed recently several sources of astrocytic glutamate release have been described, including glutamate derived from gliotransmission. However during conditions of (chemical, electrical or behavioral) stimulation a significant part of glutamate might be derived from neurotransmission. Interestingly accumulating evidence suggests that glutamate determined by microsensors is more likely to reflect basal synaptic events. This would mean that microdialysis and microsensors are complementary methods to study extracellular glutamate. Regarding GABA we concluded that the chromatographic conditions for the separation of this transmitter from other amino acid-derivatives are extremely critical. Optimal conditions to detect GABA in microdialysis samples – at least in our laboratory – include a retention time of approximately 60 min and a careful control of the pH of the mobile phase. Under these conditions it appears that 50–70% of GABA in dialysates is derived from neurotransmission.

Keywords: Microdialysis; Microsensor; GABA; Glutamate; Baclofen; TTX; TBOA; Extracellular

Contents

* Corresponding author. University of Groningen, Department of Biomonitoring and Sensoring, University Centre for Pharmacy, Antonius Deusinglaan 1, Groningen 9713 AV, The Netherlands. Tel.: +31 503633302; fax: +31 503636908.
E-mail address: m.van.de.zeyden@rug.nl (M. van der Zeyden).

0091-3057/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2007.09.004

1. Introduction

1.1. Glutamate and GABA neurotransmission

GABA and glutamate are the main inhibitory and excitatory neurotransmitters, respectively, in the central nervous system (CNS). Both neurotransmitters play an important role in the physiology of the brain, but also in various pathophysiological concepts such as depression, schizophrenia and epilepsy. In addition, these transmitters are implicated in the mechanism of a variety of centrally acting drugs.

Glutamate was first fully accepted as an amino acid neurotransmitter at the end of the 1970s. Except for being a neurotransmitter, glutamate is an intermediate in energy metabolism of the CNS, detoxifies ammonia *via* the formation of glutamine, is an important building block in the synthesis of proteins and peptides, including glutathione, is involved in fatty acid synthesis and is a precursor for GABA. For years, it has been difficult to differentiate between its role as neurotransmitter and its role in neuronal metabolism (Conti and Weinberg, 1999; Watkins, 2000). Glutamate is used by approximately 50–60% of all terminals in the CNS as a neurotransmitter (Coyle and Puttfarcken, 1993). Over half of the 100 billion neurons in the brain generate glutamate (in contrast to *e.g.* the brains 10 000 dopamine-generating neurons (McGeer et al., 1987)). Furthermore 90% of all neurons display sensitivity to glutamate. Recently it was demonstrated that glutamate can co-release in addition to monoaminergic neurotransmission (Trudeau, 2004). Glutamate is involved in most aspects of normal brain functioning, including cognitive processes and the formation of memory. It also plays a major role in neuron-development and synaptic plasticity of the CNS, including the migration, differentiation and death of cells, and in the induction and elimination of synapses. High concentrations in the extracellular fluid (ECF) caused by excessive glutamate release are associated with neuronal dysfunction resulting in a variety of neurodegenerative and psychiatric disorders including epilepsy, motor neuron disease, traumatic brain injury, Huntington's chorea, Parkinson's, Alzheimer's disease, amyotrophic lateral sclerosis and stroke (Nicholls, 1993; Danbolt, 2001). Abnormal changes in glutamate neurotransmission are also implicated in pathologies such as schizophrenia, depression, drug abuse and addiction (Palucha and Pilc, 2005).

GABA is a ubiquitous, non-protein amino acid produced through the α-decarboxylation of L-glutamic acid. GABA is the most comprehensively studied inhibitory neurotransmitter in the mammalian CNS. Brain GABA content is greater than most other neurotransmitters, occurring in 30–40% of all synapses. The neurotransmitter is most highly concentrated in the substantia nigra and globus pallidus nuclei of the basal ganglia, followed by the hypothalamus, the periaqueductal grey matter and the hippocampus. GABA is also found in spiny or aspiny granular neuronal cells, interneurons and in glial cells such as astrocytes (Bolam et al., 1983; McCormick et al., 1993; Fraser et al., 1994). GABA, which is heterogeneously distributed throughout the brain, regulates many neuronal processes and is consequently involved in many neurological conditions. GABA activates Cl^- channels termed $GABA_A$ receptors and elicits metabotropic G-protein-mediated responses by $GABA_B$ receptors. The $GABA_A$ receptor plays an important role in therapeutic applications as it is a target for drugs such as the benzodiazepines, barbiturates and certain anesthetics. Numerous studies indicate that abnormal GABA levels in ECF are associated with various disorders such as anxiety as well as Alzheimer's late cortical cerebellar atrophy, Huntington's chorea and Parkinson's disease (Raiteri et al., 2002).

1.2. Microdialysis: pros and cons

Since two decades the *microdialysis technique* (Ungerstedt, 1991; Westerink and Cremers, 2007) is the most common method to sample and detect neurotransmitters in the living brain. The method has several important advantages. Concentration *vs.* time profiles can be obtained from freely moving individual animals and information on several compounds can be obtained simultaneously. In addition the microdialysis probes can be used for local delivery of drugs to specific brain regions. Simultaneous sampling from multiple sites is possible by implanting more than one probe in the same animal. Microdialysis can be used to obtain direct measurements of free drug concentration in the brain following a systemic injection *in vivo*. The dialysis principle provides protein free samples, avoiding sample clean up procedures. With current analytical methods low detect limits are possible.

Several disadvantages of the microdialysis method are also evident. Microdialysis provides information on a time scale which

is considerably slower than many dynamic processes in the CNS, having a low temporal response, with sample collection times typically varying from 1 to 30 min (although occasionally rapid sampling times down to a few seconds have been achieved: Rada et al., 2003; Rossel et al., 2003). Another disadvantage of the method is the size of the probe (>250 μm in diameter) that will induce extensive tissue damage. The dialysis probe creates a disrupted zone of tissue as inflammation, gliosis and swollen axons were observed within 1.4 mm away from the probe (Clapp-Lilly et al., 1999). Borland et al. (2005) found that after acute implantation the release of dopamine was strongly inhibited in the vicinity of the probe, whereas the effect of reuptake inhibitors was artificially enhanced. As the dialysis probe is surrounded by scar tissue soon after implantation, the microdialysis method might represent a neuropathological rather than a neurophysiological condition. However, tissue inflammation and scar development can be reduced by making use of sterile perfusion fluids and careful swivel and tubing protocols (Zhou et al., 2002; Huff et al., 2003).

1.3. Criteria for determining neuronal release

As microdialysis probes induce extensive damage to brain tissue, the question arises whether the sampled neurotransmitter is derived from synaptic events. Therefore various criteria are used to critically evaluate the significance of neurotransmitters sampled by microdialysis. The criteria for determining neuronal release in the ECF is founded on the general functions of neurons, which are expected to be sensitive to potassium (K^+) depolarization, sodium (Na^+) channel blockage induced by *e.g.* tetrodotoxin (TTX), removal of calcium (Ca^{2+}), and depletion of presynaptic vesicles by local administration of the selective neurotoxin α-latrotoxin. For the monoamines, dopamine, noradrenaline, serotonin and acetylcholine it was found that under a wide variety of experimental conditions, dialysate contents of these transmitters indeed arise predominately from exocytotic processes (Imperato and DiChiara, 1984; L'Heureux et al., 1986; Consolo et al., 1987; Westerink et al., 1987; Kalen et al., 1998). The dialysate levels of these transmitters decrease rapidly when excitation–secretion coupled release mechanisms are inhibited (*e.g.* by calcium omission or blockade of voltage-gated sodium channels with TTX). However the neuronal origin of glutamate and GABA measured by microdialysis remains questioned, as most authors agree that glutamate does not and GABA only partly fulfill the classical release criteria for exocytotic release (Westerink et al., 1987; Westerink and De Vries, 1989; Bourdelais and Kalivas, 1992; Kehr and Ungerstedt, 1988; Osborne et al., 1990; Smith and Sharp, 1994; Timmerman and Westerink, 1997).

1.4. Microsensors an alternative?

A possible alternative to microdialysis of neurotransmitters is the use of *microsensors*. Electrochemical sensors based on amperometry have been mainly used to measure intrinsically electroactive neurotransmitters (*i.e.* catecholamines and indolamines), since these compound are readily oxidized at electrodes. Microsensors have been particularly useful in the measurement of dopamine, serotonin, purines and nitric oxide levels in both anesthetized and unanesthetized animals (Giros et al., 1996; Clement, 2004). Because the microelectrodes are 10 μm in diameter, they offer excellent spatial resolution since the tissue damage is much reduced. Moreover the response time of these sensors is often in the subsecond range.

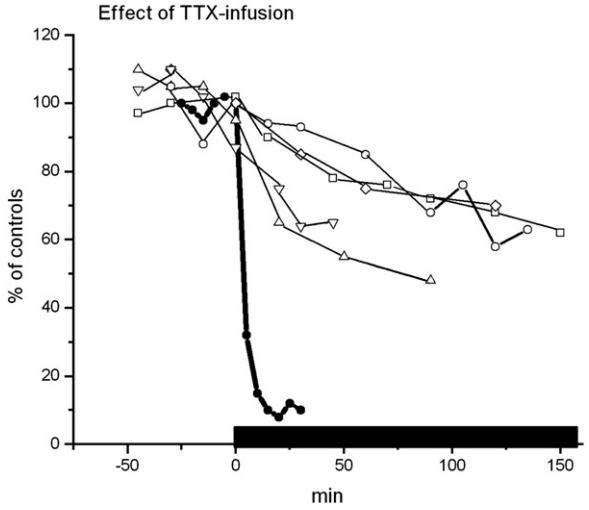

Fig. 1. Effect of 1 μM TTX infusion on extracellular levels of GABA and dopamine in the brain. Solid horizontal bar indicates the period of infusion. Results are expressed as mean±SEM% change from pre-drug baseline levels. Closed circles: dopamine, results obtained from Feenstra and Botterblom (1996). Open symbols: various studies on microdialysis of GABA, summarized in Timmerman and Westerink (1997).

For analytes that are not intrinsically electroactive (such as glutamate) specific enzymes need to be immobilized onto the surface of electrodes. A considerable number of enzymes, mainly oxidases and dehydrogenases, have been successfully incorporated into the design of electrochemical sensors. Most biosensors used in brain research use however oxidase enzymes with amperometric detection methods. Ensuing detection of the analyte can be accomplished by monitoring the consumption of an electroactive enzyme cosubstrate, such as oxygen, or the formation of an electroactive product, such as hydrogen peroxide. A cross-linkable redox polymer can be used to form a hydrogel, which both immobilize enzymes onto the microelectrode and shuttle electrons between the entrapped enzymes and electrodes (Gregg et al., 1991; Heller, 1992; Mitala and Michael, 2006). In recent years several enzyme-based electrochemical biosensors have been developed and described for monitoring hydrogen peroxide, choline, acetylcholine, glucose and glutamate *in vivo* in brain tissue of laboratory animals. Interestingly evidence is provided that microsensors have more easy access to the synaptic pools of neurotransmitters than microdialysis probes (Yang et al., 1998; Kulagina et al., 1999).

2. Microdialysis of GABA

2.1. Controversial findings on GABA in dialysates

Microdialysis is presently the most reliable method to quantify GABA in the ECF of the brain. GABA in dialysate samples is first derivatized with fluorogenic reagents such as *o*-

phtaldialdehyde, fluorescamine or naphthalene-2,3-dicarboxaldehyde and subsequently analyzed by HPLC in conjunction with fluorescence or electrochemical detection (Kehr and Ungerstedt, 1988; Westerink and De Vries, 1989; Rea et al., 2005). However the neuronal origin of GABA in microdialysis samples is debated as infusion of TTX or calcium-free Ringer solutions on GABA levels in dialysates produced inconsistent results. Calcium omission and TTX infusion have been reported to be partly effective (Osborne et al., 1990; Drew and Ungerstedt, 1991; Campbell et al., 1993; Rakovska et al., 1998), slightly effective (Stengard and O'Conner, 1994) or nonresponsive (Morari et al., 1993; Timmerman and Westerink, 1995; Ferraro et al., 2000) in reducing basal GABA levels. Fig. 1 shows that in studies reporting TTX sensitivity, GABA levels decreased very slowly, reaching only statistical significance after 30 min or more, whereas neurotransmitters such as dopamine disappear within 5 min from the dialysates (Feenstra and Botterblom, 1996). This slow decline is in strong contrast with the fast-transmitter role that GABA is believed to play in the CNS, even through it is now well recognized that GABA has slow-acting properties too, *i.e.* tonic inhibition *via* extrasynaptically located receptors.

Another controversy concerns the presence of $GABA_B$ autoreceptors in the brain. *In vitro* studies have clearly demonstrated that activation of $GABA_B$ autoreceptors by specific agonists, such as baclofen, strongly decreases GABA release as result of an inhibitory autoreceptor feedback mechanism (Lambert and Wilson, 1994; Lanza et al., 1993). However in microdialysis studies, administration of baclofen was found to have little (Boudelais and Kalivas, 1992) or no effect (Richards et al., 1998; Timmerman and Westerink, 1995) on GABA levels.

As a result of the limited response of GABA to TTX, Ca^+ omission, and selective pharmacological agents such as baclofen, many investigators have questioned the neuronal origin of GABA in microdialysates. In this regard it was suggested that GABA measured by microdialysis is derived from non-classical neurotransmission such as reverse-uptake by carrier mediated processes (Bernath and Zigmond, 1988; Pin and Bockaert, 1989) or from non-neuronal or cytoplasmic pools (Bernath and Zigmond, 1998) such as glial cells (Campbell et al., 1993; Timmerman and Westerink, 1997).

2.2. HPLC detection of GABA critically evaluated

In view of the discrepancies listed above, we have recently critically evaluated the analytical chemistry of the most frequently used method to assay GABA: derivatization of dialysates with OPA-mercaptoethanol followed by HPLC and fluorescence detection (Kehr and Ungerstedt, 1988; Westerink and De Vries, 1989).

By reducing the methanol content of the mobile phase, the retention time of GABA was increased to 60 min. During this condition several peaks co-eluted close to GABA. These peaks were not distinguishable from GABA, when the retention time was adjusted to values less than 30 min. The optimal retention time of approximately 1 h was found – in our hands – to be a prerequisite for a reliable chromatographic separation of GABA when a 15-cm long Supelcosil LC-18-DB column (4.6 mm diameter) and a flow rate of 1 ml/min was used. Furthermore the retention time of the GABA-OPA derivative appeared to be very sensitive to the pH of the mobile phase, as compared with the retention times of other amino acid-OPA derivatives such as glutamate and glycine. It was found that a change in mobile phase as small as 0.3 pH units moved the GABA peak over other unknown biological peaks (see Fig. 2). Apparently the pH dependency of the retention of GABA-OPA derivative seems to have been overlooked in the literature, as the applied pH of the mobile phase differs strongly among the various methods (pH 3.6–6.1). Even with a retention time for GABA of 60 min, only a small pH window was found (around 5.26±0.01) in which GABA was reliable separated from the co-eluting compounds.

Under these optimal chromatographic conditions suggested, basal GABA levels of 2.45±0.1, 2.95±0.5, 3.62±0.75 fmol/μl sample/mm dialysing membrane were obtained in dialysate from hippocampus, striatum and prefrontal cortex respectively. These results are lower than many values reported in the literature (range 1.6–300 fmol/μl sample/mm; $n=39$). Under the modified chromatographic conditions extracellular GABA decreased during infusion of 10 μM TTX to 35% (hippocampus; Fig. 3), 50% (striatum) and 55% (prefrontal cortex) compared to basal values. Even so, the omission of Ca^+ caused a decrease to 55 (Fig. 3), 60 and 67% of controls, respectively. This indicates that a significant part of GABA measured by the modified analytic approach is indeed of synaptic origin. Interestingly infusion of the $GABA_B$ autoreceptor baclofen decreased extracellular GABA levels in the hippocampus to about 35% of controls (Fig. 4).

2.3. On the origin of GABA in microdialysates

We propose that the complexity of the analysis of GABA-OPA derivatives might have contributed to the controversial

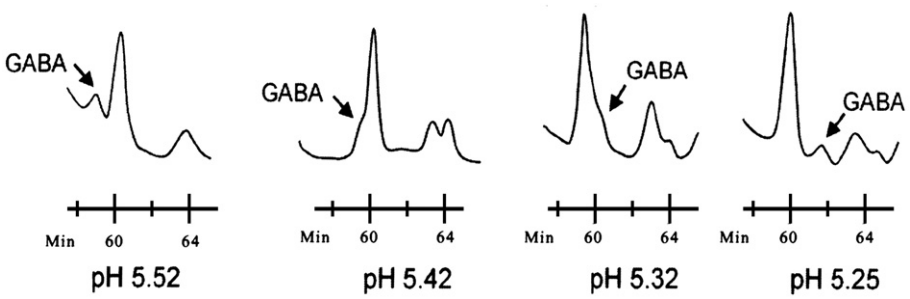

Fig. 2. The effect of minor changes in pH on the chromatographic behavior of GABA in microdialysates (Rea et al., 2005).

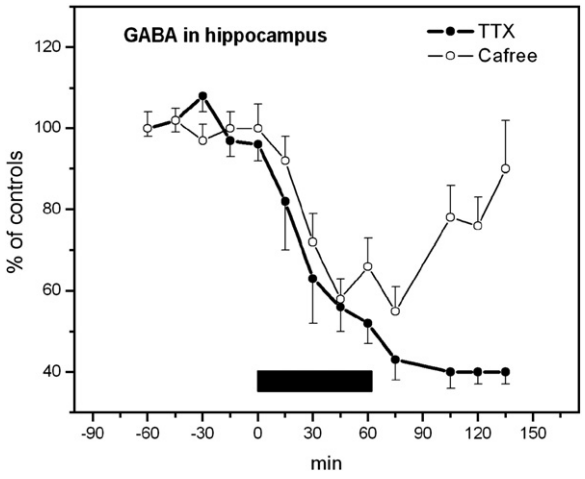

Fig. 3. Effect of infusion of 10 μM TTX ($n=10$) or Ca^{2+} free ringer ($n=6$) on extracellular levels of GABA in the hippocampus. Solid horizontal bar indicates the period of infusion. Results are expressed as mean±SEM% change from pre-drug baseline levels. GABA levels were significantly decreased by TTX infusion and calcium-free infusion. *Post hoc* test indicates significance ($p<0.05$) from 30 to 150 min after drug infusion (Rea et al., 2005).

findings of numerous microdialysis studies. Although we have demonstrated that GABA levels established during the infusion of TTX, baclofen or calcium omission consistently decreased, its response is still remarkable slow and does not go further down to 30–40% of controls. It is difficult to explain that GABA levels in microdialysates are much less responsive when compared with the "slow-acting" neurotransmitters such as dopamine (shown in Fig. 1). We propose that the microdialysis probe is in much closer contact with the overwhelming majority of glial cells and subsequently with the glial GABA pool rather than with the transmitter pool in the synaptic cleft. In other words microdialysis probes can only indirectly detect synaptic release by sampling GABA that accumulates in glial cells. We hypothesize that the GABA source in glial cells acts like a buffer that finally determines the GABA concentration in the ECF. This presence of the glial pool might explain the time delay that was observed in GABA in dialysates during conditions in which synaptic GABA release was inhibited by TTX or baclofen.

This model has important implications for interpretation of GABA in microdialysis experiments. It means that rapid decreases in GABA release – *e.g.* during behavioral experiments – might be masked by the delay (under the described conditions of about 15 min) caused by the transfer of the transmitter to the glial pool.

3. Microdialysis of glutamate

3.1. Glutamate in the ECF of the brain

Glutamate is ubiquitously expressed throughout the mammalian CNS, with intracellular concentrations in the millimolar range, in which the cytosolic concentrations is about 5 to 10 mM and the vesicular concentration approximately 0.1 M. The high-affinity glutamate transporters (EAAT1-5) assure a high signal-to-noise ratio of neurotransmission and keep the extracellular concentrations of glutamate low in order to avoid the excitotoxic actions of glutamate (Bergles et al., 1999; Amara and Fontana, 2002). Subsequently glutamate transporters are localized in the vicinity of the synapse, removing the released glutamate immediately (Diamond and Jahr, 1997; Lehre and Danbolt, 1998). Although glutamate concentration in the synaptic cleft may reach concentrations of several millimoles it has been calculated that glutamate transporters have the potency to bring concentrations in the ECF locally down to ±20 nM (Zerangue and Kavanaugh, 1996; Levy et al., 1998). Although these transporters are also present on glutamate neurons, the astroglial cells seem to play a dominant role in this respect. To assure a high signal-to-noise ratio for neurotransmission and to avoid excitotoxic actions of glutamate on neurons, the (extrasynaptic) ECF concentrations of glutamate are believed to be maintained at ±1–3 μM (Barbour et al., 1994; Diamond & Jahr, 1997; Turecek & Trussell, 2000).

To sample and determine glutamate in the ECF of conscious animals, the microdialysis method has been used in numerous studies. Glutamate in dialysates is first derivatized with fluorogenic reagents such as *o*-phtaldialdehyde, fluorescamine or naphthalene-2,3-dicarboxaldehyde and subsequently analyzed by HPLC in conjunction with fluorescence or electrochemical detection. Quantitative microdialysis studies have consistently reported ECF glutamate concentrations in the range of 1–5 μM (Miele et al., 1996; Niwa et al., 1996; Shiraishi et al., 1997; Lada et al., 1998; Zhang et al., 2004).

As the glutamate transporters are not evenly distributed over the glial membranes, it is to be expected that the glutamate concentration will also display a heterogenous concentration in the ECF. Moreover, there is accumulating evidence that astrocytes actively release glutamate which means that at certain sites in the ECF glutamate release will dominate over uptake. Consequently, different extracellular compartments of glutamate might exist in the ECF (see below).

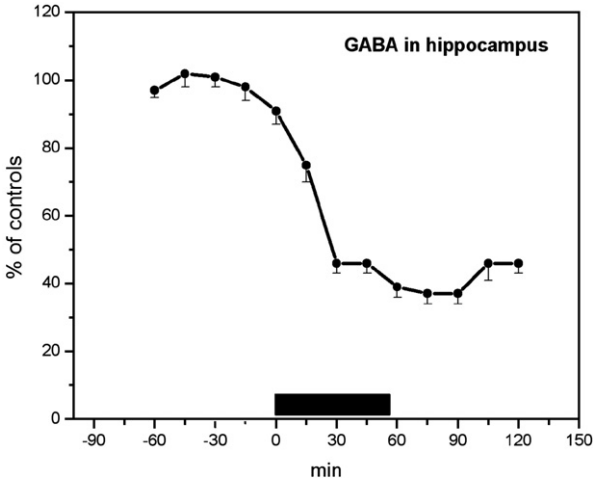

Fig. 4. Effect of infusion of 100 μM baclofen for 60 min, on the extracellular levels of GABA in the hippocampus. The solid horizontal bar indicates the period of infusion. Results are expressed as mean±SEM% change from pre-drug baseline levels. GABA levels were significantly decreased by baclofen infusion ($n=6$). *Post hoc* test indicates significance ($p<0.05$) from 30 to 150 min after drug infusion (Rea et al., 2005).

3.2. The origin of glutamate in microdialysates during basal conditions

As mentioned in the Introduction, most if not all authors agree that basal values of glutamate in microdialysis samples do not respond to TTX infusion or omission of calcium (Westerink et al., 1987; Timmerman and Westerink, 1997). One explanation for the difficulty to detect glutamate of synaptic origin in dialysates is the fact that the "spill-over" of synaptically released glutamate to the ECF is limited even though the synaptic cleft is in continuity with the extrasynaptic space. The average peak concentration of glutamate attained in the cleft has been estimated to be about 1 mM, with a time constant of decay of 1 ms (Clements et al., 1992). The highly active glutamate transporters might explain this fast mechanism (Zerangue and Kavanaugh, 1996; Levy et al., 1998). Accordingly glutamate will diffuse only very briefly into the sampling domain of the microdialysis probe. It is evident that the microdialysis probe simply due to its size has no access to the intracellular space of synapses. Another explanation for the unexpected behavior of glutamate might be related to the traumatic brain injury caused by the large dimensions (200–500 μm diameter) of the microdialysis probe. Close examination of the tissue near the implantation site of the probe has produced evidence for disturbances in microcirculation, edema, changes in the rate of glucose utilization, loss of integrity of blood brain barrier, production of cytokines and eventual proliferation of glial cells (Benveniste and Diemer, 1987; Clapp-Lilly et al., 1999; Khan and Michael, 2003; Borland et al., 2005). Scar formation around the probe might act as a barrier to diffusion which further will separate the dialysis membrane and the synapse.

3.3. Is glutamate able to escape from the synaptic cleft during excessive neuronal stimulation?

Given the fact that glutamate apparently hardly escapes from the synaptic cleft during basal conditions, various authors have attempted to detect synaptic glutamate during excessive neuronal stimulation. The latter was achieved by means of chemical, electrical or behavioral stimulation of glutamate transmission. In addition some researchers succeeded to improve the temporal resolution of the microdialysis sampling to 1 min and even seconds by using capillary electrophoresis with laser-induced fluorescence detection (Lada et al., 1997; Rossel et al., 2003).

Chemical stimulation includes the infusion of excitatory agents *via* the microdialysis probe to increase the release of glutamate. The potassium-channel blocker 4-aminopyridine prolongs the opening of the potassium channels. Infusion of this compound resulted in an increase in glutamate levels in microdialysates, which was largely TTX dependent (Peña and Tapia, 2000). Infusion of the glutamate high-affinity transport blocker DL-threo-β-benzyloxy aspartate (TBOA), resulted in a pronounced increase of glutamate (to about 300% of controls). However, this increase in glutamate was not TTX dependent (Xi et al., 2003) which indicates that glutamate accumulation also under these conditions is primarily derived from astrocytes. A different but rather successful approach to detect synaptic glutamate was to use a *dual-probe microdialysis method*. During this approach the cell-body area of glutamatergic neurons are chemically stimulated by infusion of excitatory agents *via* the microdialysis probe, and the transmitter in the terminal area is then monitored simultaneously. In this regard, application of substance P in the substantia nigra increased extracellular levels of glutamate in the striatum (Reid et al., 1990). In comparable experiments, local administration of NMDA in the frontal cortex increased glutamate levels in the striatum (Palmer et al., 1989; Dijk et al., 1995).

In several studies, *electrical stimulation* of the cell-body areas of glutamate pathways has been successfully applied to increase extracellular glutamate levels in microdialysates of the corresponding nerve terminal areas. For example, stimulation of the medulla, sciatic nerve or raphe magnus increased glutamate in areas of the spinal cord (Kapoor et al., 1990; Paleckova et al., 1992; Sorkin and McAdoo, 1993). Electrical stimulation of the nervus vagus increased glutamate levels in the nucleus tractus solitarius (Allchin et al., 1994), whereas stimulation of the nucleus paragigantocellularis increased glutamate in the ipsilateral locus coeruleus (Liu et al., 1999). Electrical stimulation of the prefrontal cortex induced an increase (in 5-s samples) of extracellular glutamate in the striatum of anesthetized rats (Lada et al., 1997). Application of a 10-s train of pulses induced a rapid increase in glutamate to 200–300% of controls that returned to basal values within 60 s. The increase in glutamate was calcium and TTX dependent. The stimulation of glutamate was suppressed by the metabotropic glutamate receptor agonist, 1-aminocyclopentane-trans-1,3-dicarboxylate (ACPD). Tucci et al. (2000) demonstrated that electrical stimulation of the prefrontal cortex increased extracellular glutamate in the nucleus accumbens.

Certain types of behavioral stimulations are accompanied by characteristic changes (often an increase) in extracellular levels of glutamate in microdialysis experiments. In several studies, extracellular glutamate was recorded during restraint stress. Substantial increases in glutamate levels to 200–450% of basal values lasting for 10–20 min were reported for the prefrontal cortex, hippocampus, nucleus accumbens and striatum (see references in Timmerman and Westerink, 1997). Photic stimulation of the conscious rat increased extracellular glutamate in the visual cortex to 200% of controls for at least 3 min (Reyes et al., 2002), and formalin injection into the hind paw resulted in an increase of extracellular glutamate that lasted for 2 min in certain subareas of the hypothalamus (preoptic area but not in lateral and ventromedial hypothalamus) (Silva et al., 2004). Results with a different time scale were presented by Rossell et al. (2003), who stimulated a whisker of the rat and sampled glutamate in the motor cortex. By using capillary electrophoresis coupled to laser-induced fluorescence detection, they were able to determine glutamate in 1-s microdialysis samples. Upon whisker stimulation, glutamate was increased only in the first 1-s sample; in the 2-s sample glutamate again fell back to control values, although the whisker stimulation was continued. The results of the 1-s lasting increase in glutamate content contrast with the 2–20-min lasting increases in

glutamate that were observed in the behavioral experiments discussed above. These data demonstrated the importance of the time scale in detecting physiological changes in extracellular glutamate. It is likely that glutamate displaying subsecond increases reflects different origin than glutamate that was enhanced in behavioral experiments lasting several minutes.

An intriguing question is whether the above-described behavioral-induced changes are calcium and TTX dependent. It was found that handling stress induced increases in glutamate that persisted in the presence of TTX (Timmerman and Westerink, 1997). Even mild activation of rats induces changes in glutamate levels in the ECF. Grooming – induced by dropping water onto the rat's snout – increased extracellular glutamate in the striatum to ±200% of controls (Miele et al., 1996). However, a similar increase was seen when the experiments were performed in the presence of TTX. In contrast, there is a report about feeding-induced increase in extracellular glutamate in the nucleus accumbens that was found to be TTX dependent (Saulskaya and Mikhailova, 2002). The study that used formalin injection into the hind paw described increases in glutamate that were both TTX and calcium dependent (Silva et al., 2004). However, the specificity of this observation is questioned by the fact that other amino acids, such as arginine and aspartate also responded in a calcium- and TTX-dependent manner.

In conclusion, evidence is provided that glutamate from synaptic sources is detectable in microdialysates collected during excessive stimulation of glutamate pathways. The behavioral experiments have produced so far the most interesting but also complex results. A rapid increase in glutamate with a time scale of 1 s was detected apart from changes in glutamate that were lasting during several minutes. It is unclear whether these different processes relate to synaptic release at the present time, as in most of the experiments, the TTX and calcium dependency of the observed effects was not consistently studied. It is tempting to speculate that changes of glutamate in subseconds time frame are related to synaptic activity, whereas changes in minutes might represent non-exocytotic release from yet unknown probably astroglial sources.

3.4. Non-exocytoxic release of glutamate from astroglial cells

The presence of a non-exocytotic extracellular pool of glutamate is well established (Herrera-Marschitz et al., 1996; Timmerman and Westerink, 1997). Several authors have suggested or provided evidence that this glutamate pool might be derived from astroglial cells by non-vesicular release. Nowadays, it is recognized that in many aspects astrocytes are similar to neurons. Astrocytes communicate with each other by changing intracellular calcium, while gap junctions connect them with one another. Ca-signals could spread between astrocytes in the form of Ca^{2+} waves. Spontaneous calcium oscillations in astrocytes can excite neighboring neurons, but neurons can also activate astrocytes. Evidence is accumulating that astrocytes release *gliotransmitters* in response to changes in intracellular calcium. This process is called *gliotransmission* (Pascual et al., 2005).

Here the different types of non-exocytotic glutamate released from glial cells that have been proposed are summarized:

(1) Glutamate release is regulated by *volume-sensitive organic anion channels* (VSOACs), also referred as volume-regulated anion channels (VRACs). Hypo-osmotic solutions activate these channels as part of the volume regulation and allow the efflux of aspartate, taurine, glutamate, chloride, and other anions in a calcium-independent manner. Glutamate behaving as a volume transmitter can facilitate by this mechanism the communication between cells not connected by synapses (Del Arco et al., 2003).
(2) Glutamate release by *GAP-junction hemichannels*. These channels release both ATP and glutamate, and their opening probability is controlled by changes in intracellular Ca^{2+} concentrations (Hansson et al., 2000; Parpura et al., 2004; Volterra and Meldolesi, 2005). Following the intracellular Ca^{2+}-wave diffusion between gap-junction-linked glial cells, Ca^{2+} can facilitate the simultaneous release of glutamate from adjacent glial cells, which consequently modifies the extracellular non-synaptic glutamate concentration in a wide region (Araque et al., 1999; Bezzi and Volterra, 2001; Haydon, 2001).
(3) Glutamate release controlled by the *purinergic $P2X_7$ receptor*. This receptor is gated by ATP and has been proposed to be involved in the release of glutamate as well as D-aspartate. It shares some properties with the GAP-junction hemichannels, including the increase opening probability at low Ca^{2+} concentration, but their pharmacology is different (Sperlagh et al., 2002; Wang et al., 2005).
(4) *Sodium-dependent high-affinity heteroexchange mechanisms* for glutamate and ascorbate, for glutamate and GABA, and for glutamate and glycine have been reported, but are poorly characterized (Bonanno and Raiteri, 1994).
(5) Glutamate release in astrocytes can be mediated by a *prostaglandin-dependent mechanism* after a rise of Ca^{2+} (Bezzi et al., 1998).
(6) *Reversal of sodium-dependent uptake* by glutamate transporters. This mechanism does not seem to occur during normal brain functioning, but is believed to be responsible for the massive release of glutamate from neurons and astrocytes after traumatic or ischemic injury to brain tissue (Benveniste et al., 1984; Katayama et al., 1990).
(7) Glutamate release coupled to the *cystine–glutamate exchanger*. This is a sodium-dependent glutamate transporter, which can operate in the reverse mode, *i.e.* cystine is exchanged for glutamate by transporting one molecule of cystine into the cell and one molecule of glutamate out of the cell. Accumulated cystine is reduced to cysteine and in turn converted to glutathione (Baker et al., 2002; Xi et al., 2003).

The fact that astrocytes can influence synaptic activity by release of glutamate points to a complex interaction between

glutamate and astrocytes. Glutamate released between neurons and astrocytes activates Ca^{2+} signaling in astrocytes, but astrocytes are responsible for the uptake of glutamate and thereby the control of the steady-state levels of extracellular glutamate. At present, the exact functional role of these processes has not been established.

3.5. Is glutamate released from astrocytes detectable in microdialysates?

The question arises whether the various mechanisms of glutamate release from astrocytes are detectable with microdialysis. Considering that non-exocytotic release of glutamate occurs *via* plasma membrane channels, glutamate will not likely respond to infusion of sodium-channel blockers (TTX). Similarly, most of the described release mechanism from astrocytes are calcium independent or make use of mobilization of intracellular calcium stores. Calcium omission during microdialysis experiments will probably not affect glutamate release from astrocytes. The use of specific channel agonists or blockers is needed for further characterization.

Baker et al. (2002) provided evidence that by using specific agonist and antagonists, the cystine–glutamate exchanger or antiporter is detectable in microdialysates. Whether this exchanger contributes to extracellular glutamate release during normal brain function, or whether it occurs when energy metabolism of astrocytes is compromised, is currently a matter of debate (Nedergaard et al., 2002; Cavelier and Atwell, 2005). Interestingly Baker et al. (2002) also demonstrated that glutamate released by the cystine–glutamate antiporter was able to modify dopamine transmission.

Other types of glutamate release from astrocytes, for example, related to VSOACs, GAP-junction hemichannels, and the prostaglandin dependent as well as the purinergic $P2X_7$ receptor mechanism have yet received little investigation in microdialysis studies.

Of particular interest is the role that mGlu receptors play in the regulation of glutamate. It is hypothesized that astrocytic mGlu receptors sense glutamate that is released during synaptic transmission to adjust its extracellular concentration by modulating uptake activity. Microdialysis studies showed that stimulation of group I or inhibition of group II mGluRs elevates extracellular glutamate levels in various brain regions (Melendez et al., 2004; Swanson et al., 2001; Baker et al., 2002). The fact that these effects were TTX independent supports the assumption that this type of release is derived from astrocytes.

4. Microsensors as alternative to detect glutamate

4.1. The principle of the microsensor

As microdialysis of glutamate provides only limited information about synaptic processes other methods with higher spatial and temporal resolution are needed. Glutamate microsensors (with a diameter of about 10 μm) might represent a promising alternative as the implantation requires at least 1000 times less volume in comparison with a 200 μm microdialysis probe. Moreover microsensors have often a much faster response time (subseconds to seconds) than microdialysis probes (seconds to minutes).

Electrochemical microsensors prepared from carbon fibers (diameter less than 10 μm) have been successfully used to study the release of catecholamines and serotonin in (sub)seconds from vesicles (Pothos et al., 2000), single cells (Kumar et al., 2001; Smith and Trimarchi, 2001) and in the awake brain (Giros et al., 1996; Stuber et al., 2005). Unfortunately glutamate cannot be directly determined by these methods as it is not electrochemically active. However when specific enzymes (*e.g.* glutamate oxidase; GluOx) are applied to a fiber, wire or ceramic needle, glutamate is converted to H_2O_2, which can be detected amperometrically. A range of biosensor designs for glutamate, based on this principle, have been described for direct monitoring of glutamate in the ECF (Hu et al., 1994; Kulagina et al., 1999; Burmeister et al., 2003; Oldenziel et al., 2004; Rahman et al., 2005).

The microsensor concept became of particular interest when Kulagina et al. (1999) reported that the hydrogel-coated glutamate microsensor was able to detect TTX-sensitive glutamate in the brain. The glutamate microsensor we use in our lab is based on this concept. Kulagina et al. (1999) optimized the glutamate microsensor by developing a sensor which utilizes an artificial redox mediator. Sensors based on this principle are described as "second generation" sensors. Importantly the use of a mediator decreases the potential needed for oxidation of the generated hydrogen peroxide resulting in a sensor with high selectivity and sensitivity.

Briefly, the hydrogel-based microsensors are constructed as follows (Kulagina et al., 1999; Oldenziel and Westerink, 2005). Carbon fibers with a diameter of 10 μM are trimmed to a length of 300–500 μM. The microsensors are prepared by coating the fiber with a five-component redox-hydrogel, in which L-glutamate oxidase, horseradish peroxidase and ascorbate oxidase are wired *via* poly(ethyleneglycol) diglycidyl ether to an osmium containing redox polymer (abbreviated as POs-EA). A thin Nafion coating completes the construction. The experiments performed *in vivo* are carried out amperometrically at a constant potential of −150 mV *vs.* Ag/AgCl. This rather low potential reduces the detection of various easily oxidizable substances endogenous to the extracellular space of brain tissue, *i.e.* the catecholamine and indolamine neurotransmitters, their acidic metabolites, urate and ascorbate. Furthermore, the potential at which these sensors operate is insufficiently negative to allow the microsensors to detect oxygen. As the enzymatic reaction occurs throughout an extensive three-dimensional network, an optimal current density is achieved which increases the sensitivity of the sensor. A disadvantage of the concept is that the diffusion of glutamate throughout the hydrogel layer decreases the response time of the sensor to several seconds.

As the microsensors are directly implanted in the brain issue, the specificity of the method should be critically evaluated. Interfering molecules can hinder the performance of the glutamate microsensor at different levels: (1) by immediate

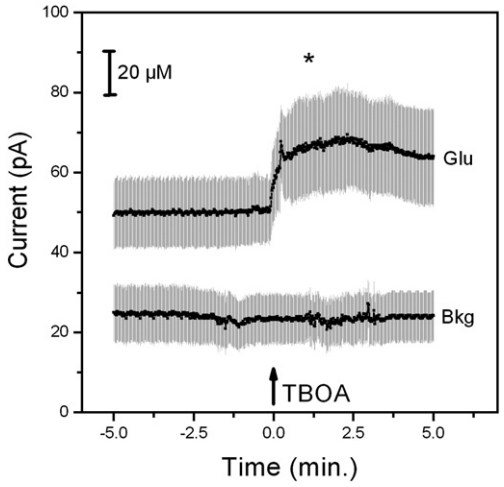

Fig. 5. Influence of TBOA on the extracellular glutamate levels. 500 nl of 1 mM TBOA ($n=7$) was locally applied and the currents produced by the glutamate (Glu) and background (Bkg) microsensors were monitored in the striatum. * denotes a statistical significant difference ($p<0.05$; Oldenziel et al., 2006).

oxidation or reduction of compounds at the carbon fiber electrode surface, (2) by interference within the redox cascade of the hydrogel and (3) by substrate aspecificity of glutamate oxidase.

These limitations caused by interfering compounds can to a certain extent be avoided by using a background microsensor. The background sensor is constructed in a similar fashion as the glutamate sensor except that glutamate oxidase is omitted from the hydrogel. The background sensor is placed in the tissue close to the glutamate sensor. When the tissue region is pharmacologically manipulated and a response at the microsensor is measured without affecting the amperometric current at the background sensor, the response can be attributed to glutamate. In this regard an interesting microsensor concept was developed in Dr G Gerhardt et al. (Day et al., 2006). Their commercially available microsensor is based on a ceramic needle like device. As this type of sensor is a first generation type, detecting glutamate (hydrogen peroxide) only at high oxidation potential, it will display a low selectivity. To overcome this disadvantage the background sensor and the glutamate sensor were attached closed to each other on the same needle. This principle is called self-referencing.

4.2. Our experience with the hydrogel-coated microsensor

Despite the fact that research on the development of a glutamate microsensor has been performed for more than a decade now, the number of studies which have successfully applied glutamate microsensors *in vivo* or *in vitro* is rather limited. This is evident by the reports of promising microsensor concepts which are not followed by routine applications (*e.g.* Hu et al., 1994; Ryan et al., 1997; Lowry et al., 1997; Kulagina et al., 1999; Mikeladze et al., 2002).

About 5 years ago we introduced the hydrogel-coated glutamate microsensor in our laboratory. The sensor based on the concept of Kulagina et al. (1999) was critically evaluated and optimized (Oldenziel et al., 2004; Oldenziel and Westerink, 2005). The next conclusions were finally drawn:

- The construction of the sensor is complex due to the fact that several individual compounds have to be cross-linked with each other to form the sensing layer. Owing to the small size of the carbon fiber, the coating of the various components is difficult to control and standardize.
- The temporal response of these glutamate microsensors appears to be determined by the diffusion of glutamate into the enzyme-containing cross-linked polymer film. The response time depends on the thickness of the layer and is relatively slow: 5–10 s.
- The sensors are still sensitive to interfering compound such as ascorbate and uric acid, and the levels of these compounds in the brain are high and difficult to control.
- Theoretically the output of the sensor is limited by oxygen deprivation. When varying the oxygen content no evidence was found for oxygen dependency (Oldenziel et al., 2006). However care should be taken when the sensor is applied at conditions in which the oxygen levels are strongly affected. For example, the well known *post-mortem* rise of glutamate that is observed during microdialysis (Geddes et al., 1999) was not detectable. In contrast in our experiments with the glutamate microsensor we observed a decrease in the glutamate sensor current after euthanasia, which is explained by deprivation of oxygen in the tissue.
- A decrease in the sensitivity of the microsensor was observed *in vivo*. This is explained by biofouling as proteins settle onto the sensor and affect sensor signal stability and disrupt the enzyme activity.
- As it is questionable whether all non-specific electrochemical signals have been eliminated, it cannot be excluded that the microsensor overestimates the glutamate concentration. Theoretically the background sensor should correct for this

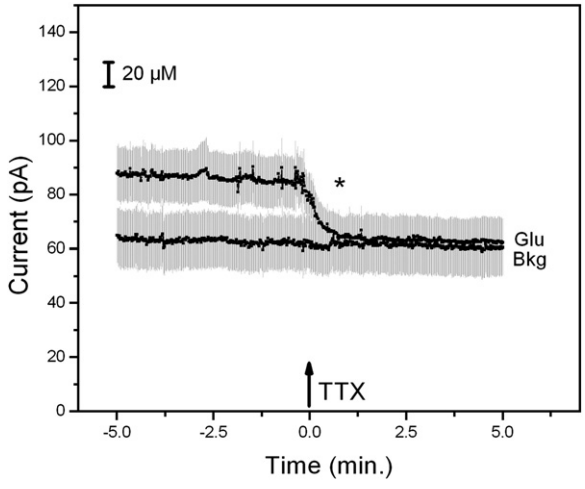

Fig. 6. Influence of TTX on the extracellular glutamate levels. 500 nl of 500 µM TTX ($n=10$) was locally applied and the currents produced by the glutamate (Glu) and background (Bkg) microsensors were monitored in the striatum. * denotes a statistical significant difference ($p<0.001$; Oldenziel et al., 2006).

error; however variation of the background sensor current can still cause misinterpretation of the signal.

Apart from these drawbacks we have successfully applied the glutamate microsensor *in vitro* as well as *in vivo* (Oldenziel et al., 2006; 2007). Basal values of the glutamate microsensor are higher than those of the background microsensor, which is indirect evidence that the microsensor is capable of detecting glutamate. Basal extracellular glutamate levels (mean±SEM) were *in vitro* 1.7±1.0 μM (CA1 area of the hippocampus; $n=19$) and *in vivo*: 18.2 μM±9.3 μM (striatum; $n=85$). This value is in good accordance with the microsensor observation reported by Kulagina et al. (1999) (29 μM), but much higher than reported by Day et al. (2006) and Rahman et al. (2005), who found values between 1.4 and 2 μM. However recently Rutherford et al. (2007), using the ceramic sensor, reported a significant difference in resting glutamate levels between the striatum (7 μM) and the prefrontal cortex (45 μM) of freely moving rats. The high values are somewhat unexpected as they are close to the excitotoxic concentrations of glutamate in the brain.

Finally the glutamate microsensor was evaluated *in vivo* in a series of pharmacological experiments carried out in anesthetized rats. In this approach compounds were administered with a micropipette connected to a pressure pulse controlled device for repeatable injections of picoliter and nanoliter volumes (PicoSpritzer®). The tip of the PicoSpritzer® was stereotactically implanted and positioned at a distance of approximately 100 μm from the glutamate microsensor.

The non-transportable reuptake inhibitor TBOA, which is a blocker of the glutamate transporters EAAT1-3 (*i.e.* GLAST, GLT-1, and EAAC1), induced a significant but rather slow increase in extracellular glutamate (Fig. 5). The gradual increase could be due to the fact that TBOA is interacting competitively with the membrane transporter. This data indicated that a constant tonic release of glutamate was present, although active release of glutamate from astrocytes by heteroexchange was not ruled out completely, as the non-transportable TBOA partly behaves as a transportable inhibitor on astrocytes (Anderson et al., 2001). The influence of TBOA on extracellular glutamate has not been investigated before with a microsensor. Interestingly the results were consistent with some recent microdialysis studies (Baker et al., 2002; Xi et al., 2003).

Importantly microinjection of TTX caused a rapid and dose-dependent decrease in the basal output of the glutamate microsensors and did not affect the background sensor (Fig. 6). This decrease suggests that – in contrast with the microdialysis method – the microsensor is indeed able to sample the synaptic pool of glutamate. These results confirm the observation by Kulagina et al. (1999), who for the first time reported that glutamate detected by microsensors was TTX-dependent. Recently, similar data were reported by Day et al. (2006) by using ceramic-based glutamate sensors. It is emphasized that for any glutamate microsensor it needs yet to be established which part of the recorded glutamate is TTX-dependent and which part is reflecting the non-synaptic pool.

5. Conclusions

5.1. GABA: analysis is critical

In various papers GABA in microdialysates respond not or only partly to infusion of TTX or the $GABA_B$ agonist baclofen or omission of calcium. By investigating the HPLC separation of GABA in conjunction with fluorometric detection, we concluded that the chromatographic conditions are very critical. Optimal conditions to detect GABA include a retention time of approximately 60 min when a 15-cm long Supelcosil LC-18-DB column (4.6 mm diameter) and a flow rate of 1 ml/min are used. Additionally the pH of the mobile phase needs to be carefully controlled. Under these conditions it appears that 50–70% of GABA is derived from neurotransmission. Apparently in several studies – including ours – the complexity of the chromatography of the amino acid-OPA derivatives was overlooked. It is evident that chromatographic protocols that determine GABA with retention times of only a few minutes are questionable. We suggest that infusion of the $GABA_B$ autoreceptor agonist baclofen (100 μM) – which decreases GABA in hippocampus dialysates to about 35% of controls – should become a standard procedure to critically evaluate the GABA analysis for both electrochemical and fluorometric assays. However even with a proper analytical approach, there is still a remarkable time delay in the TTX-induced decrease of extracellular GABA when compared to the monoamine transmitters. We hypothesize that GABA accumulated in glial cells acts like a buffer that determines the GABA concentration in the ECF.

5.2. Are glutamate microsensors an alternative to microdialysis?

Glutamate microsensors fulfill the desired criteria of improved spatial and temporal resolution. In contrast to the extensive damaged and functional disturbances that are produced by a microdialysis probe the damage caused by a microsensor is much more limited. Indeed, electron microscopy of the track created by a carbon fiber (~10 μm) revealed that maximal tissue damage was confined to a radius of 2.5 μm followed by an annular region with a width of 4 μm that contained a mix of healthy and damage cellular elements (Peters et al., 2004). This might explain why microsensors – in contrast to microdialysis probes – are able to detect TTX-dependent glutamate release during basal conditions. This result might have a significant impact on future research of extracellular glutamate. It should be emphasized that the time resolution of the glutamate sensor is limited to 5–10 s which means that only relatively slow changes (*e.g.* certain behavioral and pharmacological effects) can be recorded.

The main disadvantage of the microsensor is the complexity of its construction. This is especially true for the carbon fiber based sensors. An alternative might be the commercial available ceramic-based sensor that has a more robust construction (Day et al., 2006). However as the latter sensor has larger dimensions it needs to be established to what extent this sensor is able to detect synaptic glutamate release.

5.3. Interpretation of extracellular glutamate: two recording methods, two different sources?

Results from microsensors and microdialysis of glutamate are substantially different. First microdialysis and microsensors technologies disagree on the absolute concentrations of neurotransmitters in the ECF. Microsensors detect considerable (10–50 times) higher values. Second, microsensors – in contrast to microdialysis – are able to sample TTX-sensitive glutamate under basal conditions. The absence of any effect of TTX infusion or calcium depletion on glutamate concentrations in microdialysates under basal conditions, suggests that the source of glutamate detected with this method may represent a different pool of origin, such as astrocytic glutamate released by gliotransmission. This would mean that both methods are complementary. However additional microsensor and microdialysis experiments are needed to substantiate this hypothesis and further define the various glutamate pools that contribute to the ECF.

References

Allchin RE, Batten TF, McWilliam PN, Vaughan PF. Electrical stimulation of the vagus increased extracellular glutamate recovered from the nucleus tractus solitarii of the cat by in vivo microdialysis. Exp Physiol 1994;79:265–8.

Amara SG, Fontana AC. Excitatory amino acid transporters: keeping up with glutamate. Neurochem Int 2002;41:313–8.

Anderson CM, Bridges RJ, Chamberlin AR, Shimamoto K, Yasuda-Kamatani Y, Swanson RA. Differing effects of substrate and non-substrate transport inhibitors on glutamate uptake reversal. J Neurochem 2001;79:1207–16.

Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: glia, the unacknowledged partner. Trends Neurosci 1999;22:208–15.

Baker A, Xi ZX, Shen H, Swanson CJ, Kalivas PW. The origin and neuronal function of in-vivo nonsynaptic glutamate. J Neurosci 2002;22:9134–41.

Barbour B, Keller BU, Llano I, Marty A. Prolonged presence of glutamate during excitatory synaptic transmission to cerebellar Purkinje cells. Neuron 1994;12:1331–43.

Benveniste H, Diemer NH. Cellular reactions to implantation of a microdialysis tube in the rat hippocampus. Acta Neuropathol (Berl) 1987;74:234–8.

Benveniste H, Drejer J, Schousboe A, Diemer NH. Elevation of the extracellular concentrations of glutamate and aspartate in rat hippocampus during transient cerebral ischemia monitored by intracerebral microdialysis. J Neurochem 1984;43:1369–74.

Bergles DE, Diamond JS, Jahr CE. Clearance of glutamate inside the synapse and beyond. Curr Opin Neurobiol 1999;9:293–8.

Bernath S, Zigmond MJ. Characterization of [3H] GABA release from striatal slices: evidence for a calcium-independent process via the GABA uptake system. Neuroscience 1998;27:563–750.

Bernath S, Zigmond MJ. Dopamine may influence striatal GABA release via three separate mechanisms. Brain Res 1988;476:373–6.

Bezzi P, Carmignoto G, Pasti L, Vesce S, Rossi D, Rizzini BL, et al. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. Nature 1998;391:281–5.

Bezzi P, Volterra A. A neuron-glia signaling network in the active brain. Curr Opin Neurobiol 2001;11:387–94.

Bolam JP, Clarke DJ, Smith AD. A type of aspiny neuron in the rat neostriatum accumulates [3H] g-amino-butyric acid: combination of Golgi-staining, autoradiography, and electron microscopy. J Comp Neurol 1983;213: 121–34.

Bonanno G, Raiteri M. Release-regulating presynaptic heterocarriers. Prog Neurobiol 1994;44:451–62.

Borland LM, Shi G, Yang H, Michael AC. Voltammetric study of extracellular dopamine near microdialysis probes acutely implanted in the striatum of the anesthetized rat. J Neurosci Methods 2005;146:149–58.

Bourdelais AJ, Kalivas PW. Modulation of extracellular gamma-aminobutyric acid in the ventral pallidum using in vivo microdialysis. J Neurochem 1992;58:2311–20.

Burmeister JJ, Palmer M, Gerhardt GA. Ceramic-based multisite electrode array for rapid choline measures in brain tissue. Anal Chim Acta 2003;481:65–74.

Campbell K, Kalen P, Lundberg C, Wictorin K, Rosengren E, Bjorklund A. Extracellular gamma-aminobutyric acid levels in the rat caudate-putamen: monitoring the neuronal and glia contribution by intracerebral microdialysis. Brain Res 1993;614:241–50.

Cavelier P, Atwell D. Tonic release of glutamate by a DIDS-sensitive mechanism in rat hippocampal slices. J Physiol 2005;564:397–410.

Clapp-Lilly KL, Roberts RC, Duffy LK, Irons KP, Hu Y, Drew KL. An ultrastructural analysis of tissue surrounding a microdialysis probe. J Neurosci Methods 1999;90:129–42.

Clement P, Sarda N, Cespuglio R, Gharib A. Changes occurring in cortical NO release and brain NO-synthases during a paradoxical sleep deprivation. J. Neurochem 2004;90:848–56.

Clements JD, Lester RA, Tong G, Jahr CE, Westbrook GL. The time course of glutamate in the synaptic cleft. Science 1992;258:1498–501.

Conti F, Weinberg RJ. Shaping excitation at glutamatergic synapses. Trends in Neurosci 1999;22:451–8.

Consolo S, Wu CF, Fiorentini F, Ladinsky H, Vezzani A. Determination of endogenous acetylcholine release in freely moving rats by transstriatal dialysis coupled to a radioenzymatic assay: effect of drugs. J Neurochem 1987;48:1459–65.

Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. Science 1993;262:689–95.

Danbolt NC. Glutamate uptake. Prog Neurobiol 2001;65:1–105.

Day BK, Pomerleau F, Burmeister JJ, Huettl P, Gerhardt GA. Microelectrode array studies of basal and potassium-evoked release of L-glutamate in the anesthetized rat brain. J Neurochem 2006;96:1625–35.

Del Arco A, Segovia G, Fuxe K, Mora F. Changes in dialysate concentrations of glutamate and GABA in the brain: an index of volume transmission mediated actions. J Neurochem 2003;85:23–33.

Diamond JS, Jahr CE. Transporters buffer synaptically released glutamate on a submillisecond time scale. J Neurosci 1997;17:4672–87.

Dijk SN, Francis PT, Stratmann GC, Bowen DM. NMDA-induced glutamate and aspartate release from rat cortical pyramidal neurons: evidence for modulation by a 5-HT1A antagonist. Br J Pharmacol 1995;115:1169–74.

Drew KL, Ungerstedt U. Pergolide presynaptically inhibits calcium-stimulated release of gamma-aminobutyric acid. J Neurochem 1991;57:1927–30.

Feenstra MG, Botterblom MH. Rapid sampling of extracellular dopamine in the rat prefrontal cortex during food consumption, handling and exposure to novelty. Brain Res 1996;742:17–24.

Ferraro L, O'Connor WT, Glennon J, Tomasini MC, Bebe BW, Tanganelli S, et al. Evidence for a nucleus accumbens CCK2 receptor regulation of rat ventral pallidal GABA levels: a dual probe microdialysis study. Life Sci 2000;68: 483–96.

Frazer DD, Mudrick-Donnon LA, Macvicar BA. Astrocytic GABA receptors. Glia 1994;11:83–93.

Geddes JW, Chang NG, Ackley DC, Soultanian NS, McGillis JP, Yokel RA. Postmortem elevation in extracellular glutamate in the rat hippocampus when brain temperature is maintained at physiological levels: implications for the use of human brain autopsy tissues. Brain Res 1999;831:104–12.

Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 1996;379:606–12.

Gregg BA, Heller A. Redox polymer films containing enzymes. 1. A redox-conducting epoxy cement: synthesis, characterization, and electrocatalytic oxidation of hydroquinone. J Phys Chem 1991;95:5970–5.

Hansson E, Muyderman H, Leonova J, Allansson L, Sinclair J, Blomstrand F, et al. Astroglia and glutamate in physiology and pathology: aspects on glutamate transport, glutamate-induced cell swelling and gap-junction communication. Neurochem Int 2000;37:317–29.

Haydon PG. Glia: listening and talking to the synapse. Nat Rev Neurosci 2001;2:185–93.

Heller A. Electrical connection of enzyme redox centers to electrodes. J Phys Chem 1992;96:3579–87.

Herrera-Marschitz M, You ZB, Goiny M, Meana J, Silveira S, Godukhin OV, et al. On the origin of extracellular glutamate levels monitored in the basal ganglia of the rat by in vivo microdialysis. J Neurochem 1996;66:1726–35.

Hu Y, Mitchell KM, Albahadily FN, Michaelis EK, Wilson GS. Direct measurement of glutamate release in the brain using a dual enzyme-based electrochemical sensor. Brain Res 1994;659:117–25.

Huff JK, Bresnahan JF, Davies MI. Preliminary evaluation of several disinfection/sterilization techniques for use with microdialysis probes. Life Sci. 2003;73:275–87.

Imperato A, Di Chiara G. Trans-striatal dialysis coupled to reverse phase high performance liquid chromatography with electrochemical detection: a new method for the study of the in vivo release of endogenous dopamine and metabolites. J Neurosci 1984;4:966–77.

Kalen P, Strecker RE, Rosengren E, Bjorklund A. Endogenous release of neuronal serotonin and 5-hydroxyindoleacetic acid in the caudate-putamen of the rat as revealed by intracerebral dialysis coupled to high-performance liquid chromatography with fluorimetric detection. J Neurochem 1998;51:1422–35.

Kapoor K, Nakahara D, Blood RJ, Chalmers JP. Preferential release of neuroactive amino acids from the ventrolateral medulla of the rat in vivo as measured by microdialysis. Neuroscience 1990;37:889–900.

Katayama Y, Becker P, Taumura T, Hovda D. Massive increase in extracellular potassium and the indiscriminate release of glutamate following concussive brain injury. J Neurosurg 1990;73:889–900.

Kehr J, Ungerstedt U. Fast HPLC estimation of gamma-aminobutyric acid in microdialysis perfusates: effect of nipecotic and 3-mercaptopropionic acids. J Neurochem 1988;51:1308–10.

Khan AS, Michael AC. Invasive consequences of using micro-electrodes and microdialysis probes in the brain. Tr Anal Chem 2003;22:503–8.

Kulagina NV, Shankar L, Michael AC. Monitoring glutamate and ascorbate in the extracellular space of brain tissue with electrochemical microsensors. Anal Chem 1999;71:5093–100.

Kumar SM, Porterfield DM, Muller KJ, Smith PJS, Sahley CL. Nerve injury induces a rapid efflux of nitric oxide (NO) detected with a novel NO microsensor. J Neurosci 2001;21:215–20.

Lada MW, Vickroy TW, Kennedy RT. Evidence for neuronal origin and metabotropic receptor-mediated regulation of extracellular glutamate and aspartate in rat striatum in vivo following electrical stimulation of the prefrontal cortex. J Neurochem 1998;70:617–25.

Lada MW, Vickroy TW, Kennedy RT. High temporal resolution monitoring of glutamate and aspartate in vivo using microdialysis on-line with capillary electrophoresis with laser-induced fluorescence detection. Anal Chem 1997;69:4560–5.

Lambert NA, Wilson WA. Temporally distinct mechanisms of use-dependent depression at inhibitory synapses in the rat hippocampus in vitro. J Neurophysiol 1994;72:121–30.

Lanza M, Fassio A, Gemignani A, Bonanno G, Raiteri M. CGP 52432: a novel potent and selective GABAB autoreceptor antagonist in rat cerebral cortex. Eur J Pharmacol 1993;237:191–5.

Lehre KP, Danbolt NC. The number of glutamate transporters subtype molecules at glutamatergic synapses: chemical and stereological quantification in young adult rat brain. J Neurosci 1998;18:8751–7.

Levy LM, Warr O, Attwell D. Stoichiometry of the glial glutamate transporter GLT-1 expressed inducibly in a Chinese hamster ovary cell line selected for low endogenous Na+-dependent glutamate uptake. J Neurosci 1998;18: 9620–8.

L'Heureux R, Dennis T, Curet O, Scatton B. Measurement of endogenous noradrenalin release in the rat cerebral cortex in vivo by transcortical dialysis: effect of drugs affecting noradrenergic transmission. J Neurochem 1986;46:1794–801.

Liu N, Ho I, Rockhold RW. Contribution of glutamatergic system in locus coeruleus to nucleus paragigantocellularis stimulation-evoked behavior. Pharmacol Biochem Behav 1999;63:555–67.

Lowry JP, Ryan MR, O'Neill RD. Biosensor for neurotransmitter L-glutamic acid designed for efficient use of L-glutamate oxidase and effective rejection of interference. Analyst 1997;122:1419–24.

McCormick DA, Wang Z, Huguenard J. Neurotransmitter control of neocortical neuronal activity and excitability. Cereb Cortex 1993;3:387–98.

McGeer PL, McGeer EG, Itagaki S, Mizukawa K. Anatomy and pathology of the basal ganglia. Can J Neurol Sci 1987;14:363–72.

Melendez RI, Gregory NL, Bardo MT, Kalivas PW. Impoverished rearing environment alters metabotropic glutamate receptor expression and function in the prefrontal cortex. Neuropsychopharmacology 2004;29:1980–7.

Miele M, Boutelle MG, Fillenz M. The source of physiologically stimulated glutamate efflux from the striatum of conscious rats. J Physiol 1996;497:745–51.

Mikeladze E, Schulte A, Mosbach M, Blöchl A, Csöregi E, Solomonia R. Redox hydrogel-based bienzyme microelectrodes for amperometric monitoring of L-glutamate. Electroanal 2002;14:393–9.

Mitala JJ, Michael AC. Improving the performance of electrochemical microsensors based on enzymes entrapped in a redox hydrogel. Analytical Chimica Acta 2006;556:326–32.

Morari M, O'Connor WT, Ungerstedt U, Bianchi C, Fuxe K. *N*-methyl-D-aspartic acid differentially regulates extracellular dopamine, GABA, and glutamate levels in the dorsolateral neostriatum of the halothane-anesthetized rat: an in vivo microdialysis study. J Neurochem 1993;60:1884–93.

Nedergaard M, Takano T, Hansen J. Beyond the role of glutamate as a neurotransmitter. Nat Rev Neurosci 2002;3:748–55.

Nicholls DG. The glutamatergic nerve terminal. Eur J Biochem 1993;212:613–31.

Niwa O, Torimitsu K, Morita M, Osborne P, Yamamoto K. Concentration of extracellular L-glutamate released from cultured nerve cells measured with a small-volume online sensor. Anal Chem 1996;68:1865–70.

Oldenziel WH, Beukema W, Westerink BHC. Improving the reproducibility of hydrogel-coated glutamate microsensors by using an automatic dipcoater. J Neurosci Meth 2004;140:117−26.

Oldenziel WH, Dijkstra G, Cremers TIFH, Westerink BHC. In vivo monitoring of extracellular glutamate in the brain with a microsensor. Brain Res 2006;1118:34–42.

Oldenziel WH, Westerink BHC. Improving glutamate microsensors by optimizing the composition of the redox hydrogel. Anal Chem 2005;77:5520–8.

Oldenziel WH, Zeyden van der M, Dijkstra G, Cremers TIFH, Westerink BHC. Monitoring extracellular glutamate in hippocampal slices with a microsensor. J Neurosci Methods 2007;160:37–44.

Osborne PG, O'Connor WT, Drew KL, Ungerstedt U. An in vivo microdialysis characterization of extracellular dopamine and GABA in dorsolateral striatum of awake freely moving and halothane anaesthetized rats. J Neurosci Methods 1990;34:99–105.

Paleckova V, Palecek J, McAdoo DJ, Willis WD. The non-NMDA antagonist CNQX prevents release of amino acids into the rat spinal cord dorsal horn evoked by sciatic nerve stimulation. Neurosci Lett 1992;148:19–22.

Palmer AM, Hutson PH, Lowe SL, Bowen D. Extracellular concentrations of aspartate and glutamate in rat neostriatum following chemical stimulation of frontal cortex. Exp Brain Res 1989;75:659–63.

Palucha A, Pilc A. The involvement of glutamate in the pathophysiology of depression. Drug News Perspect 2005;18:262–8.

Parpura V, Scemes E, Spray DC. Mechanisms of glutamate release from astrocytes: gap junction "hemichannels", purinergic receptors and exocytotic release. Neurochem Int 2004;45:193–205.

Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul Y, et al. Astocytic purinergic signaling coordinates synaptic networks. Science 2005;310:113–6.

Peña F, Tapia R. Seizures and neurodegeneration induced by 4-aminopyridine in rat hippocampus in vivo: role of glutamate- and GABA-mediated neurotransmission and of ion channels. Neuroscience 2000;101:547–61.

Peters JL, Miner LA, Michael AC, Sesack SR. Ultrastructure at carbon finer microelectrode implantation sites after acute voltammetric measurements in the striatum of anesthetized rats. J Neurosci Methods 2004;137:9–23.

Pin JP, Bockaert J. Two distinct mechanisms, differentially affected by excitatory amino acids, trigger GABA release from fetal mouse striatal neurons in primary cultures. J Neurosci 1989;9:648–56.

Pothos EN, Larsen KE, Krantz DE, Liu Y, Haycock JW, Setlik W, et al. Synaptic vesicle transporter expression regulates vesicle phenotype and quantal size. J Neurosci 2000;20:7297–306.

Rada P, Mendialdua A, Hernández L, Hoebel BG. Extracellular glutamate increases in the lateral hypothalamus during meal initiation, and GABA peaks during satiation: microdialysis measurements every 30 s. Behav Neurosci. 2003;117:222–7.

Rahman A, Kwon NH, Won MS, Choe ES, Shim YB. Functionalized conducting polymer as an enzyme-immobilizing substrate: an amperometric glutamate microbiosensor for in vivo measurements. Anal Chem 2005;77:4854–60.

Raiteri L, Raiteri M, Bonanno G. Coexistence and function of different neurotransmitter transporters in the plasma membrane of CNS neurons. Prog Neurobiol 2002;68:287–309.

Rakovska A, Giovannini MG, Della Corte L, Kalfin R, Bianchi L, Pepeu G. Neurotensin modulation of acethylcholine and GABA release from the rat hippocampus: and in vivo microdialysis study. Neurochem Int 1998;33:335–40.

Rea K, Cremers TIFH, Westerink BHC. HPLC conditions are critical for the detection of GABA by microdialysis. J Neurochem 2005;94:672–9.

Reid MS, Herrera-Marschitz M, Kehr J, Ungerstedt U. Striatal dopamine and glutamate release: effects of intranigral injections of substance P. Acta Physiol Scand 1990;140:527–37.

Reyes E, Rossell S, Paredes D, Rada P, Tucci S, Gonzalez LE, et al. Haloperidol abolished glutamate release evoked by photic stimulation of the visual cortex in rats. Neurosci Lett 2002;327:149–52.

Richards DA, Lemos T, Whitton PS, Bowery NG. Extracellular GABA in the ventrolateral thalamus of rats exhibiting spontaneous absence epilepsy: a microdialysis study. J Neurochem 1998;65:1674–80.

Rossell S, Gonzalez LE, Hernández L. One-second time resolution brain microdialysis in fully awake rats. Protocol for the collection, separation and sorting of nanoliter dialysate volumes. J Chromatogr B Analyt Technol Biomed Life Sci 2003;784:385–93.

Rutherford EC, Pomerleau F, Huettl P, Stromberg I, Gerhardt GA. Chronic second-by-second measures of L-glutamate in the central nervous system of freely moving rats. J Neurochem 2007;102:712–22.

Ryan MR, Lowry JP, O'Neill RD. Biosensor for neurotransmitter L-glutamic acid designed for efficient use of L-glutamate oxidase and effective rejection of interference. Analyst 1997;122:1419–24.

Saulskaya NB, Mikhailova MO. Feeding-induced decrease in extracellular glutamate level in the rat nucleus accumbens: dependence on glutamate uptake. Neuroscience 2002;112:791–801.

Shiraishi M, Kamiyama Y, Huttemeier PC, Benveniste H. Extracellular glutamate and dopamine measured by microdialysis in the rat striatum during blockade of synaptic transmission in anesthetized and awake rats. Brain Res 1997;759:221–7.

Silva E, Hernandez L, Quinonez B, Gonzalez LE, Colasante C. Selective amino acids changes in the medial and lateral preoptic area in the formalin test in rats. Neuroscience 2004;124:395–404.

Smith PJS, Trimarchi JR. Noninvasive measurement of hydrogen and potassium ion influx from cells and epithelial structures. Am J Physiol Cell Physiol 2001;280:1–11.

Smith SE, Sharp T. An investigation of the origin of extracellular GABA in rat nucleus accumbens measured in vivo by microdialysis. J Neural Transm Gen Sect 1994;97:161–71.

Sorkin LS, McAdoo DJ. Amino acids and serotonin are released into the lumbar spinal cord of the anesthetized cat followed intradermal capsaicin injections. Brain Res 1993;607:89–98.

Sperlagh B, Kofalvi A, Deuchars J, Atkinson L, Milligan CJ, Buckley NJ, et al. Involvement of P2X7 receptors in the regulation of neurotransmitter release in the rat hippocampus. J Neurochem 2002;81:1196–211.

Stengard K, O'Connor WT. Acute toluene exposure decreases extracellular gamma-aminobutyric acid in the globus pallidus but not in striatum: a microdialysis study in awake, freely moving rats. Eur J Pharmacol 1994;292:43–6.

Stuber GD, Roitman MF, Phillips PE, Carelli RM, Wightman RM. Rapid dopamine signaling in the nucleus accumbens during contingent and noncontingent cocaine administration. Neuropsychopharmacology 2005;30(5): 853–63.

Swanson CJ, Baker DA, Carson D, Worley PF, Kalivas PW. Repeated cocaine administration attenuates group I metabotropic glutamate receptor-mediated glutamate release and behavioral activation: a potential role for Homer. J Neurosci 2001;21:9043–52.

Timmerman W, Westerink BHC. Brain microdialysis of GABA and glutamate: what does it signify? Synapse 1997;27:242–61.

Timmerman W, Westerink BHC. Extracellular gamma-aminobutyric acid in the substantia nigra reticulate measured by microdialysis in awake rats: effect of various stimulants. Neurosci Lett 1995;197:21–4.

Trudeau LE. Glutamate co-transmission as an emerging concept in monoamine neuron function. J Psychiatry Neurosci 2004;29:296–310.

Tucci S, Contreras Q, Paez X, Gonzalez L, Rada P, Hernandez L. Medial prefrontal transaction enhances social interaction. II: neurochemical studies. Brain Res 2000;887:259–65.

Turecek R, Trussell LO. Control of synaptic depression by glutamate transporters. J Neurosci 2000;20:2054–63.

Ungerstedt U. Microdialysis—principles and applications for studies in animals and man. J Intern Med 1991;230:365–73.

Volterra A, Meldolesi J. Astrocytes, from brain glue to communication elements: the revolution continues. Nat Rev Neurosci 2005;6:626–40.

Wang X, Shi F, Wosten HA, Hektor H, Poolman B, Robillard GT. The SC3 hydrophobin self-assembles into a membrane with distinct mass transfer properties. Biophys J 2005;88:3434–43.

Watkins JC. L-Glutamate as a central neurotransmitter: looking back. Biochem Soc Trans 2000;28:297–310.

Westerink BHC, Cremers TIFH, editors. Handbook of Microdialysis. Amsterdam: Elsevier; 2007. 697 pp.

Westerink BHC, Damsma G, Rollema H, de Vries JB, Horn AS. Scope and limitations of in vivo brain dialysis: a comparison of its application to various neurotransmitter systems. Life Sci 1987;41:1763–76.

Westerink BH, De Vries JB. On the origin of extracellular GABA collected by brain microdialysis and assayed by a simplified on-line method. Naunyn Schmiedebergs Arch Pharmacol 1989;339:603–7.

Xi ZX, Shen H, Baker DA, Kalivas PW. Inhibition of non-vesicular glutamate release by group III metabotropic glutamate receptors in the nucleus accumbens. J Neurochem 2003;87:1204–12.

Yang H, Peters JL, Michael AC. Coupled effect of mass transfer and uptake kinetics on in vivo microdialysis of dopamine. J Neurochem 1998;71:684–92.

Zerangue N, Kavanaugh MP. Flux coupling in a neuronal glutamate transporter. Nature 1996;383:634–7.

Zhang FF, Wan Q, Li CX, Wang XL, Zhu ZQ, Xian YZ, et al. Simultaneous assay of glucose, lactate, L-glutamate and hypoxanthine levels in a rat striatum using enzyme electrodes based on neutral red-doped silica nanoparticles. Anal Bioanal Chem 2004;380:637–43.

Zhou F, Braddock JF, Hu Y, Zhu X, Catellani RJ, Smith MA, et al. Microbial origin of glutamate, hibernation and tissue trauma: an in vivo microdialysis study. J Neurosci Methods 2002;119:121–8.