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A Glass Capillary-based Microsensor for L-Glutamate in *in vitro* Uses

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1. Introduction

Since the pioneering work by R. Adams (1976), who detected a neurotransmitter catecholamine in mammalian brain by implanting a solid carbon electrode directly in animal brains, a lot of miniaturized *in vivo* and *in vitro* sensors for neurotransmitters have been poposed (Hirano&Sugawara, 2006; Sugawara, 2007; Zeyden et al. 2008; O'Neill et al., 1998). The field is slowly, but continually, expanding. Most of researches aim at developing electrochemical sensors with spatial and temporal resolution, which enable us to discern distribution of neurotransmitters within each neuronal subfield and estimate its concentration level and temporal changes in intact brains, acute slices and cultured neurons.
In the central neuronal system of mammalian brain, L-glutamate is released from synaptic terminals and plays a vital role in brain development, synaptic plasticity, neurotoxicity, and neuropathological disorders (Reis et al., 2009; Bliss&Collingdige, 1993; Malenka&Nicoll, 1999). L-Glutamate is also involved in neuropathological disorders such as epilepsy, stroke, Parkinson's disease and Alzheimer's disease (Nishizawa, 2001; Mattoson et al., 2008). L-Glutamate may activate transmitter receptors located extrasynaptically on neurons and glia at greater distance from the place of exocytosis of synaptic vesicles, though the concentration level of such spillover of L-glutamate from synaptic cleft is not clear yet (Volterra&Meldolesi, 2005). The basal and enhanced level of extracellular L-glutamate plays a key role in neuronal functions, because its level will determine whether L-glutamate has actions or negligible actions on most glutamate receptors (Herman&Jahr, 2007).
Up to date, the *in vivo* level of L-glutamate in brain has been reported mostly for corpus striatum, while no *in vivo* data have been reported for the hippocampus. On the other hand, acute slices of hippocampal tissue offer experimental control of the neuronal network environment. In the *in vitro* case as well, a very limited number of microsensors have been applied to acute brain slices, probably because of technical difficulties in handling thin living slices (thickness 200-400 μm) and the lack of suitable miniaturized sensors.
A glass capillary-based enzyme sensor has been developed in which a three-electrode system is built in the capillary (Nakajima et al., 2003), hence outer reference and auxiliary electrodes are not necessary to be set in brain tissue. The sensor with the tip diameter of approximately 10 μm is promising as a microsensor for monitoring the enhanced extracellular level of L-glutamate release in each neuronal region of acute hippocampal slices

under chemical and electric stimulation. In this review, we describe the principle, properties and application of a glass capillary-based sensor for *in vitro* monitoring of L-glutamate in hippocampal slices.

2. Preparation and response principle of a glass capillary sensor for L-glutamate

2.1 Preparation of a glutamate oxidase (GluOx)-coated Au electrode

A working Au electrode, which is to be set in a capillary pipette, is prepared in the conventional manner (Oka et al., 2007). The one end of a gold wire (ø 0.30 mm) is coated with 0.3 μl of a detergent solution supplied as refill kit peroxidase (Os-gel-HRP, Bioanalytical systems, USA). The gold is then coated twice with each 0.3 μl of the Os-gel polymer followed by air-drying overnight. The surface of the Os-gel-HRP is coated with 1 μl of ACSF (Mg^{2+}, Ca^{2+}-free) solution containing 2% bovine serum albumin (BSA), 0.2% glutaraldehyde and 65 U/ml GluOx. The electrode is necessary to be stored at 4˚C until use. In this protocol, Nafion coating and ascorbate oxidase immobilization, which are commonly used for eliminating interference from L-ascorbate, are not employed, because the inner solution of a glass capillary sensor contains L-ascorbate oxidase (*vide infra*).

2.2 Preparation of a capillary sensor

The structure and photo of a glass capillary microsensor is shown in Fig. 1. The capillary sensor is composed of a Borosilicate glass capillary (outer diameter 1.5 mm and inner diameter 0.86 mm) having a tip diameter of approximately 10 μm, prepared by using a three-pull technique with a micropipette puller. The tip diameter can be measured under a microscope. Before use, the glass capillary is filled with a Mg^{2+}, Ca^{2+}-free ACSF (approximately 3 μl) containing $1x10^3$ U/ml ascorbate oxidase (AsOx). A Teflon-coated Pt wire (ø 0.127 mm) with ~2 mm of exposed Pt and a Teflon-coated Ag/AgCl wire (ø 0.127 mm) served as counter and reference electrodes, respectively. The working, counter and reference electrodes are inserted into the capillary pipette. The distance between the tip of the glass pipette and the working electrode is usually ~1.5 mm or shorter, as observed under a microscope. A larger distance leads to a delay in the response to L-glutamate.

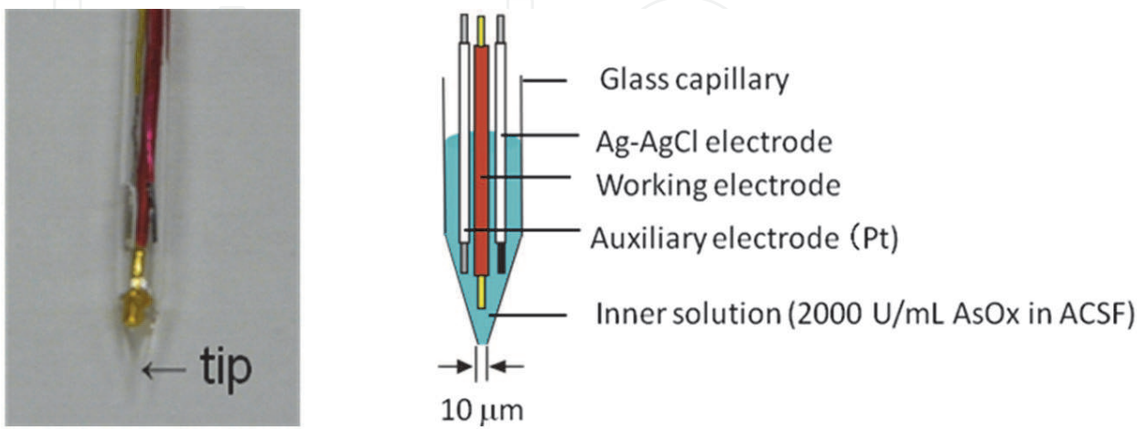

Fig. 1. A photo of a glass capillary sensor and its structure.

2.3 Electrochemical reaction at a glass capillary sensor

L-Glutamate released in brain slices diffuses into the inner solution of a capillary sensor to reach the top layer (GluOx-BSA layer) of the underlying working electrode. L-Glutamate oxidase (GluOx) catalyzes the oxidation of L-glutamate into α–ketoglutarate, producing electro-active H_2O_2 (Kusakabe et al., 1983).

$$\text{L-glutamate} + H_2O + O_2 \rightarrow \ \alpha \text{ ketoglutarate} + NH_4^+ + H_2O_2 \quad (1)$$

The Os-gel-HRP on the working electrode mediates the reduction of H_2O_2 in the following way (Vreeke et al., 1992).

$$2Os(II) + H_2O_2 + 2H^+ \rightarrow 2Os(III) + 2\,H_2O \quad (2)$$

$$Os(III) + e^- = Os(II) \quad (3)$$

The Os(III) produced is reduced at the underlying electrode, giving a reduction current, which is used as a response of the present sensor. The operation potential is 0 V vs. Ag-AgCl.

The capillary electrode has the advantage that the inner solution can contain various enzymes. The interference from ascorbic acid, one of the major components in the brain, is removed by adding ascorbate oxidase to the inner solution. The enzyme catalyzes the oxidation of L-ascorbate to 2-dehydroascorbate (Tokuyama et al., 1965; Nakamura et al., 1968) according to

$$\text{L-ascorbate} + 1/2\,O_2 \rightarrow \text{dehydroascorbate} + H_2O \quad (4)$$

The pH range (pH 5.6-7.0) for the catalytic action of ascorbate oxidase is very close to that (pH 5.5-10.5) of GluOx (Kusakabe et al., 1983) , and hence both enzymes are active at pH 7.0.

2.4 Monitoring L-glutamate with a capillary sensor

Our protocol for monitoring L-glutamate in brain slices is as follows. Prior to its implantation into a hippocampal slice, a glass capillary sensor is operated in air at 0 V vs. Ag-AgCl until a steady current is obtained. Then, the sensor is positioned above the surface of a target neuronal region of a hippocampal slice in interface preparation, followed by lowering into the target region of the slice at a depth of ~100 μm with a manipulator. Appearance of a sharp electric noise indicates the implantation of the sensor into the slice. The sensor is kept in the slice until a steady current is attained. After attainment of a steady current, recording an L-glutamate current is started. Since the volume of the sensor inner solution is maintained, continuous and long-time monitoring of L-glutamate in a brain slice is feasible.

3. Response principles of a capillary sensor in bulk solutions and brain slices

The response profiles of a capillary sensor are categorized into two cases, depending on whether it is used in an aqueous solution just above the target region of a brain slice or it is implanted in the target region of a brain slice (Sugawara, 2007). When a capillary sensor is positioned in a bulk aqueous solution just above a brain slice, capillary action is essentially important for its operation. In an aqueous solution, a small volume of a sample solution

containing L-glutamate is spontaneously sampled into an inner solution by capillary action. On the other hand, such capillary action does not work in brain slices because of viscous nature of extracellular fluid. It is noted that a small fraction of GluOx is leached from the top surface of a GluOx-immobilized Au electrode into a capillary inner solution (Oka et al., 2007) and hence, leached GluOx catalyzes the oxidation of L-glutamate, producing hydrogen peroxide, which is detected at the working electrode.

3.1 Capillary action of a pulled glass capillary in an aqueous solution

Figure 2 shows the photos that demonstrate the capillary action of a pulled glass capillary in an aqueous solution. The capillary inner solution contained a visible dye, i.e., methylene blue(MB). One can see that an aqueous solution comes into the capillary with time. When an aqueous solution contained 5% dextrane, the sampling rate was deteriorated significantly due to an increase in viscosity. In another set of experiments, we quantified the capillary action by measuring the weight of a capillary dipped in an aqueous solution (Nakajima et al., 2003). With a tip diameter of 2.5 μm or less, a rise of the solution by capillarity is not observed. On the other hand, in the case that a capillary with a tip diameter of 10 μm is dipped in an aqueous solution, the weight of the solution in the capillary increases linearly with dipping time up to 20 min. The slow rise of the solution is due to the conical tip of the capillary, which decelerates the movement of the solution. Thus, pulled glass capillaries exhibit different magnitudes of capillarity, which depend on tip size and dipping time. The quantitative response of a glass capillary sensor in an aqueous solution is relied on such capillary action.

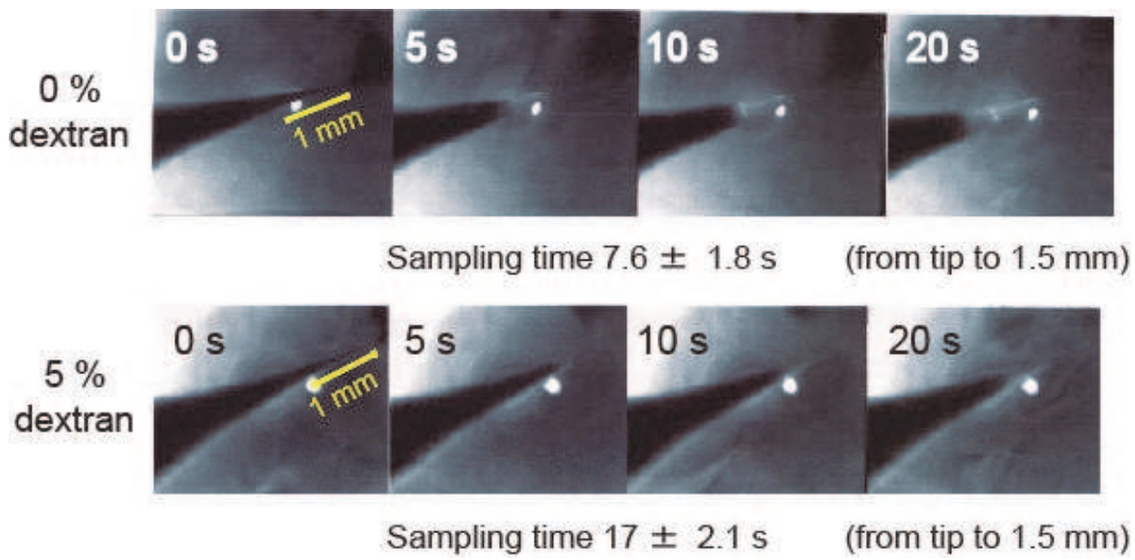

Fig. 2. Photos that demonstrate capillarity-based sampling of an aqueous solution. The inner solution of a glass capillary contained methylene blue.

3.2 Diffusion of L-glutamate into a capillary inner solution

In contrast to its use in an aqueous solution, the capillary action of a glass capillary does not work in a brain slice because of the viscous nature of extracellular fluid. The volume of an inner solution of a glass capillary is maintained, as shown in Fig. 3, even after its

implantation into a brain slice. Under such circumstance, the response of a capillary-based sensor is based on diffusional entry of L-glutamate into its inner solution.

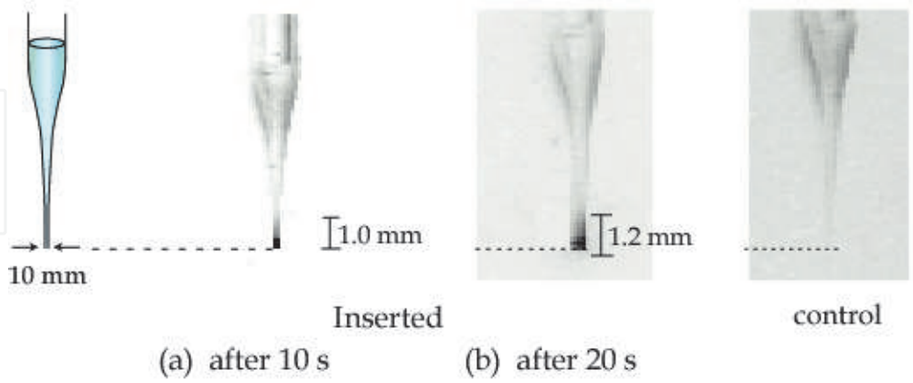

Fig. 3. Fluorometric images of a glass capillary. A pulled glass capillary containing ACSF (Mg^{2+} and Ca^{2+}-free) was inserted into a brain slice, loaded in advance with a fluorescence dye BCECF (Oka et al., 2007).

3.3 *In situ* calibration

One of the essential tasks to be considered is how to correlate the sensor response to final L-glutamate concentration. There are two ways for maintaining brain slices alive (Sugawara, 2007), *i.e.*, a brain slice is fully submerged in a bath solution (Fig. 4a) and a slice is kept alive by passing an ACSF underneath the slice (Fig. 4b). Calibrating the implanted glass capillary sensor is also dependent on how slices were maintained. In the submerged case, calibrating sensor responses and stimulation of the slice can be performed by changing the concentration of L-glutamate or a stimulant in the bath solution. For brain slices in interface preparation, and also in submerge preparation, post-*in vitro* calibration is common for calibrating the responses of an implanted sensor, because the adsorption of extracellular components on the top surface of a sensor deteriorates the sensitivity of the response. In this protocol, an implanted sensor is transferred into an aqueous solution and calibrated with a standard L-glutamate solution. However, the post-in vitro calibration approach is based on the assumption that the sensor exhibits the same degree of deterioration both in a brain slice and an aqueous solution. To improve the uncertainty of the post-*in vitro* calibration, we suggested a method for calibrating an implanted sensor by injecting a small volume of (5 μl) of a standard L-glutamate solution into the close vicinity of the glutamate sensor through a glass capillary (Oka et al., 2007; Chiba et al., 2010). The sensor exhibits a transient current-time profile rather than a steady one (Fig. 5), due to the active reuptake process and diffusional wash out of L-glutamate. Consequently, an instantaneous current is used for calibration. The calibration has to be done at each neuronal region, because the activity of reuptake process is neuronal region-dependent.

It is noted that L-glutamate levels measured with a capillary sensor are dependent on the type of slice preparation (Sugawara, 2007). In the submerged case where a sensor is positioned above the surface of a target neuronal region, an L-glutamate level obtained is the one that diffused out of the slice. Such alignment of a sensor is common for not only capillary sensors but also patch sensors using natural receptors. However, thus-obtained L-

glutamate levels are obviously lower than those in the brain slice (Oka et al., 2009). Consequently, a relative change in the response rather than the very magnitude of the response is a matter of concern for monitoring neuronal events. On the other hand, the implantation of a sensor into a brain slice can measure L-glutamate in the vicinity of neurons, but calibrating the sensor response needs a hard task.

The lower detection limit for L-glutamate of GluOx-based sensors has been reported to be sub-μM or better. The detection limit of nM range has also been reported (Tang, et al., 2007; Braeken et al., 2009). However, these values are based on the measurements in an electrolyte solution rather than in brain or brain tissues. The properties of tissue environment, for example viscosity, differ significantly from an electrolyte solution. The diffusion of L-glutamate affected by viscosity may alter the sensitivity of the sensor. Therefore, *in situ* calibration of the sensor response in brain tissue is important for knowing the detection limit of an implanted sensor.

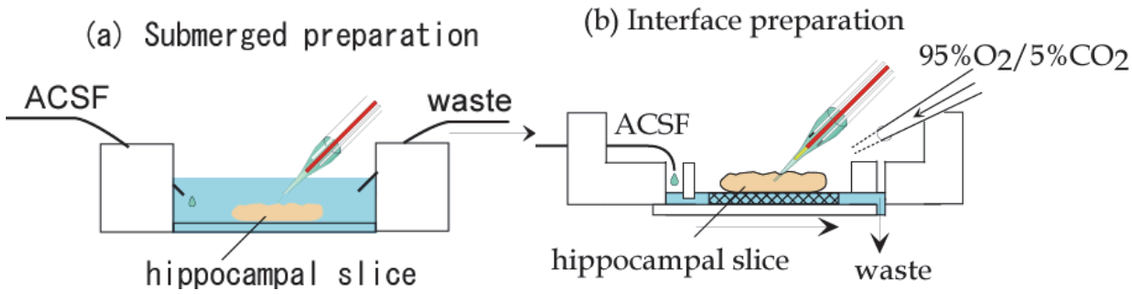

Fig. 4. Two types of slice preparation. (a) A brain slice is submerged in a bath solution and (b) a brain slice is placed on a lens paper through which an ACSF flows (Sugawara, 2007).

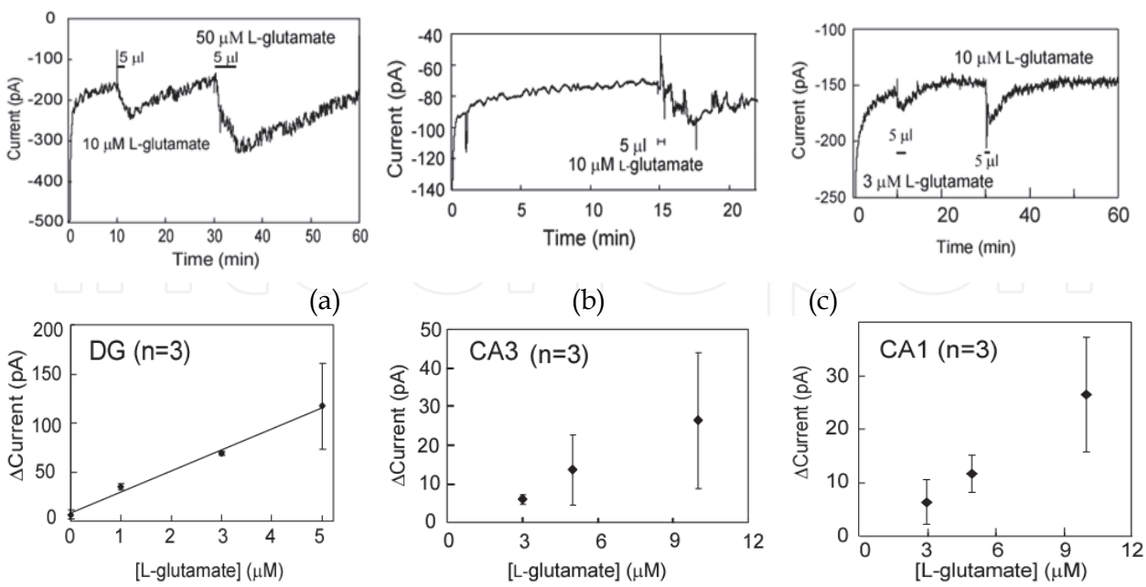

Fig. 5. Current-Time profiles for *in situ* calibration at (a) DG , (b) CA3 and (c) CA1 and corresponding calibration graphs for L-glutamate (Oka et al., 2007, 2009; Chiba et al., 2010).

3.4 Selectivity

A number of interfering species coexist with glutamate in brains. The selectivity issues of GluOx-based sensors for eliminating interference from coexisting ions and molecules have been addressed by several authors. Interference from ascorbate that are present in the brain at concentration much larger than that of L-glutamate has been eliminated by coating Nafion that excludes ascorbate anions electrostatically from the electrode surface (Day et al., 2006; Oldenziel et al., 2006a; Rutherford et al., 2007; Burmeister &Gerhardt, 2001] or by using ascorbate oxidase that mediate the decomposition of ascorbate before it approaches to the surface of an underlying electrode (Oldenziel et al. 2006b; Kulgina et al, 1999; Oka et al. 2007) . Conducting polymer-modified electrodes are also effective for eliminating the interferent (Rahman et al., 2005). Another potential interfering compound is L-glutamine. The interference arises probably from the presence of glutaminase as contaminant in isolated GluOx (Yamauchi et al., 1984). The isolated GluOx-based sensor shows a response to glutamine at a few μM level. However, at large glutamine concentration above 300 μM, which corresponds to the concentration of glutamine in brain (Kanamori&Ross, 2004; Lerma et al., 1986), the response to glutamine is saturated. In addition, such interference disappears in the presence of a small amount of L-glutamate (Oldenziel et al., 2006).

The response to glutamine is significantly modified by using recombinant GluOx (Hozumi et al., 2011). The recombinant GluOx-based sensor suffers interference from glutamine only at high concentration above 300 μM, and the response to glutamine is very weak (1.83 pA/μM an average between 300 and 500 μM) in comparison with the response to L-glutamate (472 pA/μM). The glutamate sensor based on recombinant GluOx does not exhibit responses to glycine, GABA, serotonin, (each 1.0 mM), and L-aspartic acid (200 μM). Since the typical concentration of L-ascorbic acid in brain is 100-500 μM (Walker et al., 1995; Nedergaard et al., 2002) and the estimated basal concentration of glutamine in brain ranges from 200 to 400 μM (Lerma et al., 1986; Kanomori and Ross, 2004) and that of L-aspartic acid is 0.25 μM or less (Robert et al., 1998), the effect of these compounds on L-glutamate currents appears to be of the minor importance.

4. Monitoring of L-glutamate release in hippocampal slices

Since L-glutamate released from nerve terminals into the synaptic cleft is subject to diffusion and dilution into extracellular space and uptake into neurons and glia by excitatory amino acid transporters, the extracellular level of L-glutamate is essentially important for elucidating neuronal signal transmission processes. Although the *in vivo* level of L-glutamate in the neuronal subfield of the hippocampus is important (Table 1), no reports have been published yet on hippocampal levels of L-glutamate. A small number of data have been gathered from *in vitro* study using acute hippocampal slices that consist of freshly isolated brain tissue maintained in a chamber. The data described in this section are mostly taken from our results. The extra-cellular and extra-slice L-glutamate levels in acute mouse hippocampal slices obtained with a capillary sensor are given in Table 2, together with the reported basal levels for hippocampal slices (Oldenziel et al, 2007; MaLamore et al., 2010, Hermann&Jahr, 2007). The reported basal level varies in a wide range from several tens nM to a few μM (Table 1). Therefore, the basal L-glutamate level in brain and brain slices is still a matter of debate.

4.1 Chemical stimulation

The monitoring of enhanced L-glutamate level evoked by physiologically relevant stimuli enables us to discern the role and action of each stimulant as well as the regional distribution of L-glutamate in hippocampal slices. We performed monitoring of L-glutamate release in various neuronal regions of mouse hippocampal slices under stimulation with KCl, tetraethylammonium (TEA) chloride and ischemia.

4.1.1 KCl stimulation

The depolarization evoked by KCl (0.10 M) stimulation enhances extracellular L-glutamate level in hippocampal slices, but the enhanced concentration level at dentate gyrus (DG) (Oka et al., 2007) is much larger than those at cornu ammonis 1 (CA1) (Oka et al., 2009) and cornu ammonis 1 (CA3) (Chiba et al., 2010). The K^+-evoked L-glutamate levels in the CA1 and CA3 regions are very low, i.e., approximately 4 μM, owing to reuptake processes. Sodium-dependent excitatory amino acid transporters (EAATs) (Taxt et al., 1984; Rothstein et al., 1994; Furuta et al., 1997) are present differentially within neurons and astroglia (Taxt et al., 1984): EAAC1(EAAT3) is highly enriched in hippocampus with distribution of CA1, CA3 > DG, while EAAT4 is present in trace amount in hippocampus (Furuta et al., 199). The regional transporter distribution suggests that L-glutamate in the CA1 and CA3 regions is more strongly removed from extracellular space than in the DG region and hence, the L-glutamate level is maintained very low.

4.1.2 TEA stimulation

The stimulation by a K^+ ion channel blocker tetraethylammonium (TEA) chloride is known to elicit chemically induced synaptic potentiation (cLTP) in CA1 of hippocampal slices (Aniksztejn&Ben-Ari, 1991; Hosokawa et al., 1995). The TEA stimulation activates both NMDA receptor channels (Hanse&Gustafsson, 1994) and voltage-dependent calcium channels (Huang et al., 1993). The activation of the NMDA receptor channels induces a calcium influx, often inducing LTP, which is similar to that evoked by a brief afferent tetanus (electrical stimulation). The regional distribution of extracellular L-glutamate in hippocampal slices under TEA stimulation (Oka et al., 2007; Oka et al., 2009, Chiba et al., 2010) is similar to that obtained by K^+ stimulation, though L-glutamate levels at CA1 and CA3 are slightly larger than those obtained by K^+ stimulation.

4.1.3 Ischemia

One of the most enigmatic aspects of ischemic injury is the selective vulnerability of the hippocampal CA1 neurons, whereas the neurons in the CA3 and DG regions are relatively spared. The monitoring of L-glutamate with a glass capillary sensor showed that the extra-slice level of L-glutamate is in the order of CA1 ≈ CA3 > DG (Nakamura, et al., 2005), which is in accordance with the imaging study in terms of an L-glutamate flux (Hirano et al., 2003; Okumura et al., 2009). The concentration level of L-glutamate is much larger than those observed by other chemical stimulation (Table 2), indicating that the high level of L-glutamate is released into the extracellular space and diffuses out of the slice into the bath. The time course of the L-glutamate flux at region CA1 is biphasic and that at region DG is modestly biphasic. Similar biphasic time course of L-glutamate release has been reported for rat striatum (vulnerable to ischemic injury) by using a dialysis electrode (Asai et al., 1996; Kohno et al., 1998).

Region	Concentration (μM)	Method	Size (μm)	Reference
Striatum	29.0 ± 9.0	carbon fiber E	10	Kulagina et al 1999
Brain	23.6 ± 5.3	hydrogel E	10	Oldenziel et al. 2006a
Striatum	18.2 ± 9.3	hydrogel E	10	Oldenziel et al. 2006b
Striatum	7.3 ±0.9	self-referencing E	15	Rutherford et al. 2007
dosal striatum	2.0 ± 0.5	conducting polymer E	25	Rahman et al, 2005
Frontal cortex	1.6 ± 0.3	self-referencing E	15	Day et al. 2007
left striatum	1.5 ± 0.3	microsensor	3.0 (mm)	Zhang et al. 2004
striatum	1-3	ceramic-based multi E	15	Pomerleau et al. 2003
right striatum	3.0 ± 0.6	microdialysis		Miele et al. 1996
striatum	2.7 ± 0.9	microdialysis		Robert et al. 1998
striatum	1.15 ± 0.7	microdialysis		Lada et al. 1997
striatum	1.97 ± 0.7	push-pull perfusion		Kottergoda et al. 2002

Table 1. Examples of basal extracellular L-glutamate levels in the anesthetized rat brain (*in vivo*).

Stimulation	Subfield (concentration, μM)	Method (size , μm)	Reference
Extracellular(acute)			
KCl (0.1M)	DG (22) > CA3 (4.1) ~ CA1 (4.0)	capillary E (10)	Nakajima et al .,2003
			Chiba et al. ,2010
TEA (25 mM)	DG (20) > CA3 (7.5)~ CA1 (6.0)	capillary E (10)	Oka et al., 2009
			Chiba et al., 2010
Electric (2 Hz)	CA1 (1.4)	capillary E (10)	Hozumi et al., 2011
Electric (0.052Hz)	CA1 (32 nM)		
Basal	CA1 (1.7)	hydrogel E (10)	Oldenziel et al., 2007
Basal	CA1 (～25 nM)	patch sensor (whole cell)	Hermann and Jahr, 2007
Extracellular(cultured)			
KCl (0.1M)	hippocampal slice (0.12)	microdialysis	Robert et al. 1998
Extra-slice (acute)			
Basal	P19 cell (5.8)	self-referencing E (2-5)	Maclamore et al. 2010
GABA	CA1(1.5) > CA3	patch sensor (2)	Shimane et al. 2006
Ischemia	CA3 (60) ~CA1 (47) > DG(20)	capillary E (10)	Nakamura et al. 2005

Table 2. Regional distribution of extacellular and extra-slice levels of L-glutamate in mouse hippocampal slices.

4.2 Electric stimulation

Recording field excitatory postsynaptic potentials (fEPSPs) have been a well established method for knowing neuronal activities in electrophysiological studies (Bliss et al., 2007). The potentials are produced by a group of cells and reflect in an indirect way the changes in the synaptic and action potentials. Analysis of fEPSPs provides information on the average activity of the neurons in group, including the induction and expression of long-term potentiation (LTP) and long-term depression (LTD). Simultaneous monitoring of L-glutamate release and fEPSPs evoked by physiologically relevant electric stimulation will provide explicit information on the actual amount of L-glutamate released into the synaptic cleft and extracellular space in the vicinity of the stimulation site. However, such a measurement is still a challenging task, because of the requirement of placing multiple electrodes in a confined neuronal region and fairly low level of glutamate will be released by physiologically relevant stimulation.

A glass capillary sensor has the advantage that all electrodes required for amperometric current measurements are built in the capillary interior and hence, the tip size（approximately 10 μm）is small enough to implant it between stimulation and recording electrodes for fEPSP measurements. Fig. 6 shows the setup of simultaneous recordings of a glutamate current with a glass capillary sensor and fEPSPs with stimulation and recording electrodes. The tip of the capillary sensor can be positioned in the middle between stimulation and recording electrodes for fEPSP measurements. The example of simultaneous measurements at CA1 of a hippocampal slice demonstrates that although no significant changes in a glutamate current is detected by application of 0.052 Hz (test stimuli), a transient change in the current is observed by application of 2 Hz stimulation, indicating enhanced release of L-glutamate in CA1 region (Hozumi et al., 2011). The L-glutamate level in region CA1 at 2 Hz stimulation obtained by *in situ* calibration ranged from 0.8 to 2.2 μM (1.4 μM as an average) from 5 independent measurements. The concentration is slightly smaller than those obtained by KCl stimulation. Although direct monitoring of L-glutamate level at test stimuli (0.052 Hz) is not achieved, we can record an L-glutamate current by changing the intensity of electric stimulation from 1 Hz to 3 Hz. The estimated concentration level at 0.052 Hz is 32 ± 7 nM (n=3), which is very close to the reported one using the whole cell recordings (Herman&Jahr, 2007).

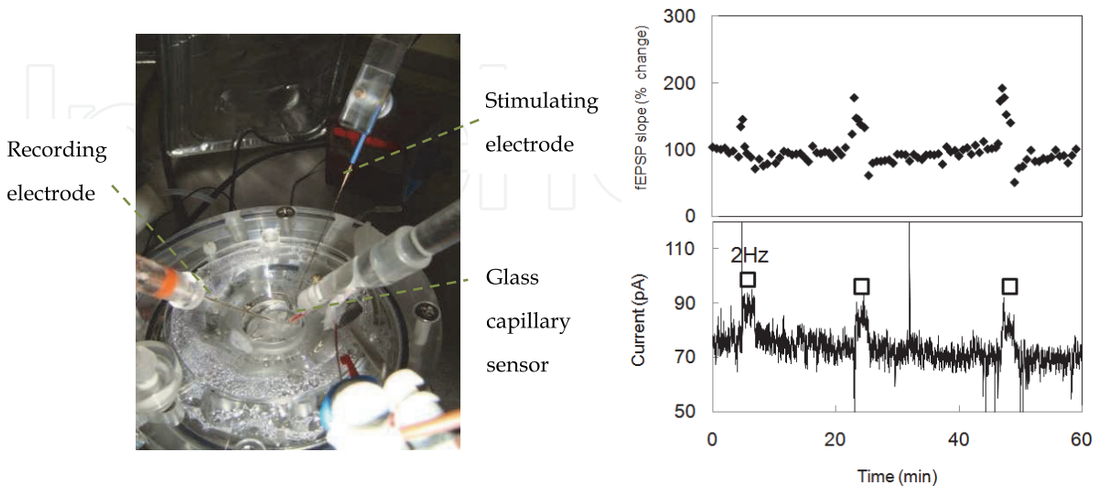

Fig. 6. A photo that shows the setup of simultaneous monitoring of fEPSPs and a glutamate current. The traces of fEPSP and a glutamate current were simultaneously monitored.

5. Conclusions and prospects

The present review demonstrates that a glass capillary-based microsensor is useful for knowing the distribution and level of extracellular L-glutamate in acute hippocampal slices. The L-glutamate levels are markedly dependent on the neuronal regions and types of stimulation. Although discerning the concentration level of L-glutamate in acute brain slices with microsensors is still on the stage of accumulating the local concentration level of L-glutamate, there are increasing efforts for clarifying the sources and places of extracellular L-glutamate release. Since the experimental condition can be controlled easily, microsensors will be promising as a tool for monitoring and estimating the averaged extracellular level of L-glutamate in acute brain slices. On the other hand, recent advances in developing new fluorescent probes have enabled to visualize L-glutamate with spine-sized resolution using a cultured neuron. Such a single synapse study will significantly help the understanding of the molecular events that occur at a synapse. Combining the single synapse data with the extracellular data will significantly advance the understanding of the sources and places of extracellular L-glutamate release. In addition, the sophisticated combination of microsensor studies with an electrophysiological study to correlate L-glutamate level to the neuronal activity will be a promising way for solving debates as to whether LTP is due to presynaptic or postsynaptic changes.

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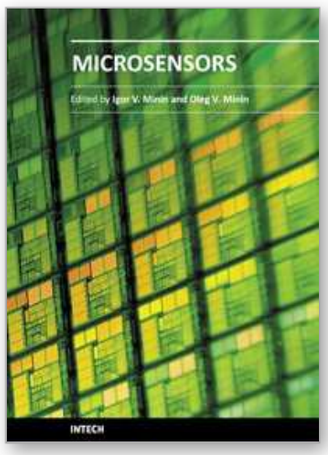

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This book is planned to publish with an objective to provide a state-of-art reference book in the area of microsensors for engineers, scientists, applied physicists and post-graduate students. Also the aim of the book is the continuous and timely dissemination of new and innovative research and developments in microsensors. This reference book is a collection of 13 chapters characterized in 4 parts: magnetic sensors, chemical, optical microsensors and applications. This book provides an overview of resonant magnetic field microsensors based on MEMS, optical microsensors, the main design and fabrication problems of miniature sensors of physical, chemical and biochemical microsensors, chemical microsensors with ordered nanostructures, surface-enhanced Raman scattering microsensors based on hybrid nanoparticles, etc. Several interesting applications area are also discusses in the book like MEMS gyroscopes for consumer and industrial applications, microsensors for non invasive imaging in experimental biology, a heat flux microsensor for direct measurements in plasma surface interactions and so on.

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