Monitoring extracellular glutamate in the rat brain by microdialysis and microsensors
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1. Introduction: neuronal–glial circuits
The brain is the most complex organ of the human body. It is composed of several highly specialized and heterogeneous populations of cells, represented by neurones (e.g. motorneurons, projection neurons or interneurons), and glia represented by astrocytes, oligodendrocytes and microglia. The neuronal doctrine, which from the 1890s shaped the development of neuroscience (von Waldeyer 1891) is constantly giving way to more inclusive studies demonstrating close bidirectional communication of neurons and glia of structural and functional levels (Haydon 2001; Volterra and Meldolesi 2005; Perea 2005). In particular, the excitatory transmitter glutamate has been shown to evoke a variety of responses in astrocytes and oligodendrocytes.

2. L-Glutamate as principle neurotransmitter
L-Glutamate, discovered in 1908 was found as a flavour and named ‘umami’ meaning “yumminess” in Japanese. The excitatory action of glutamate in the mammalian brain and spinal cord is only known since the 1950s (1954 by Hayashi). In the 1960s glutamate was introduced as a neurotransmitter and in the late 1970s, it became widely recognized as the main excitatory neurotransmitter within the vertebrate nervous system.

Glutamate satisfies the criteria to classify it as an excitatory neurotransmitter seeing that glutamate is: (i) synthesized by enzymes within neurones, (ii) present in nerve terminals, (iii) stored in presynaptic vesicles and released in a calcium dependent manner, (iv) able to bind specific receptors throughout the nervous system and exert an effect in the post-synaptic neuron, (v) inactivated via high affinity reuptake mechanisms after its release, (vi) similar to chemicals that act as glutamate receptor agonists.

Part of the reason for the delay in recognizing glutamate as an excitatory neurotransmitter, is the multiple roles that glutamate play in brain metabolism. Glutamate is the precursor to the inhibitory neurotransmitter GABA, it detoxifies ammonia within the brain and is the amino acid building block for the synthesis of proteins, peptides and metabolites such as glutathione. Much (approximately 2/3) of the brain energy demand relates to glutamate homeostasis; this includes the reuptake and recycling of glutamate. As such, functional imagining modality, such as PET and fMRI indexing activity is primarily of brain glutamatergic systems (Javitt 2004). The blood-brain barrier essentially prevents the entry of glutamate into the CNS, and therefore most of the glutamate that is present in the brain, is synthesized de novo by astrocytes (Nedergaard et al. 2002).
Glutamate, which is the reduced form of glutamic acid, is the most common excitatory neurotransmitter in the brain. Accounting for roughly 60% of neurons and 40% of synapses (Javitt 2007). Glutamate acting at the synaptic level is also intimately linked to synaptic plasticity, neuronal development and degeneration. Glutamate has been implicated in both acute and chronic pathological states such as the neuronal damage associated with brain trauma as well as that with chronic neurodegenerative diseases, such as Alzheimer’s disease, post traumatic stress disorder, schizophrenia, depression and anxiety (Meldrum et al. 1994).

3. Glutamate pathways in the brain
Prominent glutamatergic pathways (Figure 1) in the brain are; (i) the cortico-cortical pathways (pathways between the two hemispheres); (ii) the thalamocortical (pathways between the thalamus and the cortex); (iii) and the extrapyramidal pathway (the projections between the cortex and striatum). Other glutamate projections exist between the cortex, substantia nigra, subthalamic nucleus and pallidum (Carlsson 1995; Javitt 2004).
The interaction between glutamate and dopamine show special emphasises for Chapter 4, 5 and 6 in the content of this thesis. This is of great importance, since glutamate and dopamine interacts in the motive/reward circuit (Figure. 2) which plays a key in translating incoming stimuli into a behavioural response. A central component of the circuitry that mediates reward and motivation is the mesoaccumbens system which consists primarily out of a dopaminergic projection. A second critical pathway originating in the VTA is the mesoprefrontal pathway, which sends dopamine projections to the PFC. A portion of these neurons synapses directly onto glutamatergic pyramidal neurons projection to the NAc. In addition to the PFC, the NAc receives glutamatergic afferents originating in the hippocampus, mediodorsal thalamus, and basolateral amygdale (Ikeda et al. 2003).

Figure 2: Simplified schematic of the circuitry of the mesolimbic dopamine system in the rat brain highlighting the major inputs to the nucleus accumbens (NAc) and ventral tegmental area (VTA) (glutamatergic projections, blue; dopaminergic projections, red; GABAergic projections, orange). Glutamatergic synapses excite postsynaptic neurons and GABAergic synapses inhibit postsynaptic neurons. Dopamine release exerts more complex modulatory effects. AMG, amygdala; BNST, bed nucleus of the stria terminalis; LDTg, laterodorsal tegmental nucleus; LH, lateral hypothalamus; PFC, prefrontal cortex; VP, ventral pallidum (Kauer and Makenka 2007).
4. Glutamate neurotransmission

Classically, glutamate is released in the synaptic cleft at excitatory synapses in the CNS when action potentials depolarize synaptic terminals and glutamate-filled synaptic vesicles containing 400–5000 molecules of glutamate fuse with the presynaptic membrane (De Belleroche and Bradford 1977; Bergles et al. 1999). In the very small space of the synaptic cleft (±2 attoliters), concentrations of glutamate as high as several millimolar are attained, in order to assure activation of postsynaptic glutamate receptors and therefore depolarization of the postsynaptic neuron. The average peak concentration of glutamate attained in the cleft has been estimated to be about 1 mM with a time constant decay of 1 ms (Bergles et al. 1999). Accordingly chemical communication with a high signal-to-noise ratio for neurotransmission is completed avoiding the excitotoxic actions of glutamate and neurons. Glial and neuronal re-uptake then removes glutamate from the synaptic cleft to terminate the glutamate signal.

The temporal and spatial extent of glutamate release shapes the resulting response. Factors that determine the spatiotemporal profile of the concentration of glutamate in the synaptic cleft and perisynaptic space involves the dynamics of glutamate release, diffusion, binding and uptake (Bergles et al. 1999). Receptor occupancy in turn dictates the size of the postsynaptic response as well as the number of unbound receptors available for activation by subsequent exocytosis (Bergles et al. 1999). The difference between concentration decay time constants of 0.5 ms and 1.5 ms could make the difference between low receptor occupancy and nearly saturated receptors (Bergles et al. 1999).

5. Glutamate transporters and glia Na\(^+\) homeostasis

Presynaptic released glutamate is not metabolized in the extracellular space; instead, the clearance of glutamate in the synaptic cleft depends on passive diffusion and active uptake by glutamate transporters localized in the vicinity of the synapse (Lehre and Danbolt 1998; Danbolt 2001). Interestingly from the bulk of glutamate release during synaptic transmission, about 20% is accumulated into postsynaptic neurons and the remaining 80% is taken up by perisynaptic astrocytes (Swanson 2005).

Glutamate transporters are located in the plasma membrane of both neurons and glial cells and are driven by the electrochemical gradients of K\(^+\) and Na\(^+\) across the plasma membrane. Glutamate release at synapses is inactivated by five excitatory amino acid transporters (EAATs) in glia and postsynaptic neurons. EAAT1 (GLAST; glutamate-aspartate transporter) and EEAT2 (GLT1- glutamate transporter 1) are predominantly...
expressed in astrocytes, whereas EAAT3,-4,-5, are expressed in neurons (Sims and Robinson 1999). GLT-1 is the most dominant and widely distributed transporter in the CNS. It is located on the surface of astrocytes and provides 90% of glutamate uptake (Haugeto et al. 1996). Glutamate transporters tightly control the extracellular glutamate concentrations in order to ensure a high signal-to-noise ratio during synaptic transmission and prevent neuronal damage that might occur as a result of excessive activation of glutamate receptors. It has been calculated that glutamate transporters have the potency to bring concentrations of extracellular glutamate locally down to ±2 nmol (Cavelier et al. 2005).

Although these transporters are present on glutamate neurons and astrocytes, the astroglial cells seem to play a more dominant role in this respect. GLT and GLAST are more active in clearing glutamate relative to the cloned neuronal transporters which is evident by the capacity of glutamate uptake. Glial cells have 15 000 - 21 000 transporters per µm$^3$ tissue, compared with 2 000 for neuronal transporters (Dehnes et al. 1998). The need for this capacity, to clear glutamate is evident when considering that individual vesicles may contain 400–5000 molecules of glutamate and that the average synapse has ±20 release sites that can be refilled every 10 sec (Stevens and Tsuijimoto 1995). When taken into account that the glutamate transporters are not evenly distributed over the glial membranes, it is to be expected that the glutamate concentration will differ in the various extracellular compartments.

Early models of transmitter clearance indicate that passive diffusion appears to be an important mechanism for glutamate removal from the synaptic clefts on the millisecond timescale. However this is only an efficient mechanism at synapses with small diameters (a few hundred nanometers). Besides diffusion alone is not sufficient for clearance because ultimately the ambient level of extracellular glutamate would slowly rise to toxic levels. The only rapid way to remove glutamate from the extracellular fluid surrounding the receptors is by cellular uptake (Huang and Bergles 2004). The high-capacity glutamate transporter uptake system is the mechanism responsible for the long-term maintenance of low extracellular concentration of glutamate (Huang and Bergles 2004).

6. Glutamate receptors
Glutamate receptors play pivotal roles in neuronal excitation in the mammalian CNS. Glutamate receptors are membrane proteins, which mediate excitatory transmission on the cellular surface through initial binding of glutamate (Folbergrová et al. 2005). Glutamate receptors have been divided into two broad categories: ionotropic glutamate
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receptors (iGluRs) and metabotropic glutamate receptors (mGluRs) (Folbergrová et al. 2005; Rosa et al. 2005). iGluRs contain cation-specific ion channels as a component of their protein complex, while mGluRs are coupled to G-protein and modulate intracellular second messenger systems. According to the most simplistic view, these two families of glutamate receptors have distinct roles in neuronal activity. Direct and fast information transfer is mediated by postsynaptic iGluRs, whereas mGluRs either tune neuronal excitability at the postsynaptic level or control neurotransmitter release at the presynaptic level.

iGluRs and mGluRs are located both within the synaptic cleft as well as extrasynaptically. The extrasynaptic glutamate receptors can modulate the release of an impressive array of neurotransmitters including glutamate, GABA, substance P, dopamine, and noradrenaline (Cartmell and Schoepp 2000).

Presynaptic glutamate receptors act either as autoreceptors or heteroreceptors. Autoreceptors are receptors located on presynaptic nerve cell terminals of glutamatergic neurons and serves as a part of a feedback loop in signal transduction. In this regard it is relevant that mGluRs are described on glutamatergic neurons on extrasynaptic sites. Heteroreceptors comprise presynaptic receptors located on both non-glutamatergic terminals and glutamatergic terminals other than the ones that released the glutamate. Receptors that are located at a considerable distance from the presynaptic active zone (pre-terminal receptors) generally act as heteroreceptors (Pinheiro and Mulle 2008).

It is now clear that the situation in glutamate transmission is not simple and that both iGluRs and mGluRs can act presynaptically. Although presynaptic GluRs probably control the strength of synaptic transmission by altering the likelihood that synaptic vesicles will be released in response to an incoming action potential, the cellular and molecular elements that are involved in the activity of presynaptic glutamate receptors and their mechanism of action are highly variable (Pinheiro and Mulle 2008).

Basically glutamate receptors play an important role in neurotransmission, neuroplasticity and neurotoxicity in the CNS (Xi et al. 2003). Neuroplasticity concerns the modulation of glutamatergic synaptic transmission underlying memory and learning and the formation of neural networks during development (Ozawa et al. 1998). Excessive stimulation of glutamate receptors can cause excitotoxic neurodegeneration by extensive increase of intracellular Ca\(^{2+}\) levels followed by the formation of reactive oxygen species. On the other hand, several malfunctions, like ischemia induces an excessive,
pathological-like release of glutamate and thereby enforce a feedback loop which will facilitate the development of excitotoxic events (Castillo et al. 2005). Hence glutamatergic transmitter release might enhance neurodegeneration after a stroke or hypoglycaemia (Castillo et al. 2005).

Figure 3: The glutamate synapse indicating receptors and vesicles releasing glutamate into the synaptic cleft. An astrocyte with its receptors are also indicated to the left.

6.1 Ionotropic glutamate receptors (iGluR)
Ionotropic glutamate receptors are ligand-gated cation-specific ion channels. They allow the passage of charged ions into the neuron, for example Na$^+$ and Ca$^{2+}$. Based on their binding affinities for prototypical ligands or their pharmacological and electrophysiological properties, they are further categorized as N-methyl-D-aspartate (NMDA) receptors, α-amino-3-hydroxy-5-methyl 4-isoxazol-propionatne (AMPA) and kainate (KA) receptors (Rosa et al. 2005).

AMPA en KA receptors
The AMPA and the kainate (KA) receptors are collectively termed ‘non-NMDA’ receptors and appear to control conductance of Na$^+$ and K$^+$ through channels that exhibit rapid kinetics.
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Of the three ionotropic receptors in the CNS, AMPA receptors occur at the greatest densities (Tsapakis and Travis 2002). The AMPA receptors located within the synapse most often cluster in the postsynaptic membrane which has a channel permeable to monovalent cations (Na\(^+\) and K\(^+\)) and which provides the majority of inward current for generating synaptic responses when the cell is close to its resting membrane potential (Malenka and Nicoll 1998). However AMPA receptors are not only located within the synapse, but are also found on extrasynaptic neuronal and glial processes. Of all receptors AMPA receptors are the most common in glia, being detected in various astroglial cells throughout the brain, in white matter oligodendrocytes, and in NG2 glia as well as microglia (Verkhratsky and Kirchhoff 2007). AMPA receptors are composed of combination of GluR1-4 subunits, encoded by distinct genes. AMPA receptors in rat neurons are activated by glutamate concentrations between 0.1 and 10 mM (Featherstone and Shippy 2008). It is unclear how these extrasynaptic localized AMPA receptors with their mM affinity would be directly stimulated by extrasynaptic glutamate levels which are estimated to be in the low micromolar range (Patneau and Mayer 1990).

The agonists acting at AMPA receptors are AMPA and quisqualic acid. Antagonists include the competitive antagonists ATPO, DNQX, ZK 200775, NS1209 and YM-90K and the non-competitive antagonists GYKI53655 and CP-465022.

The role of KA receptors is not fully understood but as mentioned, they participate in the fast excitatory transmission. KA receptors are predominantly presynaptic and appear to regulate glutamate release. The KA receptors are composed of five distinct subunits, GluR5-7 and KA1 and KA2 subunits. KA receptors are activated at slightly lower glutamate concentrations than AMPA receptors, with EC\(_{50}\)s around 300 to 800 µM (Featherstone and Shippy 2008).

KA receptor agonists include kainate and domoic acid. CNQX and 2,3 benzodiazepine GYKI52466 are known as selective and non-selective antagonists at the AMPA/kainate receptor respectively.

NMDA receptors
The most structurally complex glutamatergic receptor is the NMDA receptor (Figure 4). It is an ion channel made up of different and variably assembled protein isoforms. It is the only ionotropic receptor to control Ca\(_{2+}\) conductance in addition to the conductance of Na\(^+\) and K\(^+\). When the channel is activated there is an influx of Na\(^+\) and Ca\(_{2+}\) ions and an efflux of K\(^+\) ions. NMDA receptors are blocked in a voltage sensitive manner by
magnesium (Mg\(^{2+}\)), which binds to a site within the NMDA ion channel. As a result, NMDA receptors are uniquely voltage as well as ligand (glutamate)-sensitive. Thus activation of the channel can only occur if there is simultaneous glutamate (and glycine; see below) binding as well as a partial depolarisation of the membrane potential, permitting Mg\(^{2+}\) displacement.

**NMDA subunits**

NMDA receptors are encoded by seven genes, NR1, NR2A-D and NR3A and B. The NMDA receptors include at least one NR1 subunit and one or more modulatory subunit(s), labelled NR2A-NR2D. NR1 subunits are synthesized to excess in neurons, but are retained in the endoplasmic reticulum until they assemble with NR2 subunits. Present data indicates that the subcellular distribution may be determined by the subunit composition. NMDA receptors localized in the synapse consists of NR1/NR2A subunits and are characterized by rapid offset kinetics while extrasynaptic localized receptors consists of NR1/NR2B subunits and exhibit slow kinetics (Tovar and Westbrook 1999).

**Modulatory sites**

Glycine and D-serine are ‘obligatory co-agonists’ for glutamate, acting on a strychnine-insensitive allosteric modulatory site of the NMDA receptor. This glycine-binding site, like the benzodiazepine site of the GABA\(_A\) receptor, regulates channel opening time and densitization rate in the presence of the agonist (glutamate), but does not, of itself, induce channel opening (Javitt 2007).

Interestingly administration of exogenous glycine and/or D-serine potentiates NMDA receptor mediated neurotransmission *in vivo*, suggesting that the glycine sites are not saturated under physiological conditions. Glycine may have a lower affinity at the glycine site of the NMDA receptor than D-serine by several previous reports. Therefore D-serine is likely the major endogenous agonist for the strychnine-insensitive glycine-modulatory site of NMDA receptors (Schell *et al.* 1995). The glycine sensitivity varies according to NR2 subunit, with NR2A-containing NMDA showing less sensitivity to glycine than those containing NR2B subunits. Because of differential saturation under basal conditions, exogenous administered glycine or D-serine may affect NR2A containing receptors to a greater extent than those containing NR2B subunits (Javitt 2004).

It was found that D-cycloserine, an antituberculosis drug, fortuitously crossreacts with the glycine site. However, D-cycloserine functions only as a partial agonist, producing only 40-60% of the response seen with either glycine or D-serine. Attempts to modify glycine
or D-serine to produce synthetic glycine site agonists have, proven to be unsuccessful thus far.

NMDA receptors exhibit a 100-fold higher affinity for glutamate than AMPA/kainate receptors (Zheng et al. 2003). In the presence of the co-agonist glycine, both native and recombinant NMDA receptor are activated by 0.5 to 50 µM of glutamate, with most EC₅₀s between 2-4 µM (Featherstone and Shippy 2008). Several studies have demonstrated that NMDA receptors can be stimulated by both vesicular and nonvesicular glutamate (Jabaudon et al. 1999; Angulo et al. 2004; Cavelier and Attwell 2005). Several other studies confirm extrasynaptic NMDA receptors as auto- or heteroreceptors capable of regulating the release of glutamate, GABA and monoamines (Wang et al. 1992; Malva et al. 1994).

![Schematic model of the NMDA receptor complex](image)

Figure 4: Schematic model of the NMDA receptor complex, showing the binding sites for glutamate, glycine and D-serine (Javitt 2006).

NMDA, quinolinic and ibotenic acid are selective agonists, whereas AP5 is a competitive antagonist and phencyclidine (PCP), the NMDA analogue dizocilpine (MK-801) and ketamine are selective uncompetitive antagonists at this receptor. Uncompetitive and competitive NMDA receptor antagonists show distinct electrophysiological, neurochemical and behavioural profiles. Although they bind to the same receptor
complex, they have different sites at the complex; that is, competitive antagonists bind to the NMDA receptor itself, whereas uncompetitive antagonists interact with a site in the receptor channel.

Excitatory postsynaptic currents (EPSCs) in most mammalian CNS have a fast AMPA receptor mediated component lasting a few milliseconds and a slow NMDA receptor mediated component, lasting hundreds of milliseconds. That’s because the voltage dependence of NMDA receptors has the effect of enhancing the depolarisation initiated by non-NMDA receptor channels. Ions like $\text{Ca}^{2+}$ can subsequently act as a second messenger and initiate a wide range of intracellular responses that underlie a number of complex neurophysiologic phenomena. Transduction through the NMDA receptor produces consequently a slow but sustained physiological response. For this reason both NMDA and AMPA receptors are essential for brain functioning in normal and diseased states. Usually NMDA and AMPA receptors are co-localized (Nusser 2000). Interestingly Takumi (1999) found that the number of NMDA receptors is positively correlated to the diameter of synapses, whereas the number of AMPA receptors is positively correlated to synaptic area, which in turn has a second power correlation with diameter. Thus the ratio of AMPA to NMDA receptors is linearly related to synaptic diameter (Nusser 2000).

**NMDA in neurodevelopment and neurodegeneration**

It is not surprising that in addition to its role in glutamatergic transmission, the NMDA receptor is also involved in neural development and activity-dependent synaptic plasticity which is thought to assist in the development of the formation of memory and learning (Hardingham and Bading 2003). There is also growing evidence that physiological levels of synaptic NMDA receptor activation promotes the survival of many types of neurons or render them more resistant to trauma (Hardingham and Bading 2003).

On the other hand, too much NMDA receptor activity is harmful to neurons – but so is too little. Blockade of NMDA receptors during the prenatal period can induce apoptosis in vulnerable neurons (Meldrum 2000) and the complete absence of NMDA receptor activity is deleterious to the cell (Hardingham and Bading 2003). By contrast when NMDA receptors are intensely or chronically activated they are associated with cell death in many neuropathological conditions. Therefore NMDA receptor activity is thought to contribute to the aetiology of many chronic neurodegenerative disorders, such as motor neuron disease (MND), or amyotrophic lateral sclerosis (ALS), Huntington’s disease, HIV associated dementia, Parkinson disease and Alzheimer’s disease (AD). It is thought that certain aspects of these disorders render neurons particularly vulnerable to endogenous
levels of NMDA receptor activity or unable to carry out normal glutamate homeostasis (Hardingham and Bading 2003).

6.2 Metabotropic glutamate receptors (mGluR)
mGluRs are a heterogenous family of G-protein –coupled receptors that are widely distributed throughout the CNS. As mentioned their function is not to mediate but rather to modulate brain excitability via presynaptic, postsynaptic and glial mechanisms (Folbergrová et al. 2005). mGluRs are perhaps the most likely of all receptors to receive tonic stimulation by extrasynaptic glutamate levels. mGluRs exhibit a very high affinity for glutamate that is comparable with NMDA receptors (Conn and Pin 1997;Anderson and Swanson 2000). But unlike NMDA receptors they are not subjected to voltage-dependent magnesium blockade. Measured EC$_{50}$s range from 0.02 µM (for mGluR8) to 1000 µM (for mGluR7), although most EC$_{50}$ values from most mGluR subtypes are ±10 µM (Featherstone and Shippy 2008).

mGluRs have seven transmembrane domains, and being G-protein coupled, they act similar to most dopamine, serotonin and noradrenaline receptors, activating phospholipase C or inhibiting adenylate cyclase. mGluRs are classified into eight subtypes, which can be categorized into three groups based on sequence homology, biochemical signalling, and pharmacological properties; Group I (mGluR1 and 5), II (mGluR 2 and 3) and III (mGluR 4,6,7 and 8) (Conn and Pin 1997;Anwyl 1999). Group I receptors are functionally linked to phospholipase C (PLC) and synthesis of 1,4,5-inositol-triphosphate (InsP$_3$) and diacylglycerol (DAG), and seems to augment ionotropic receptor function in glutamate neurotransmission (Bordi and Ugolini 1999). Group II and III inhibit adenylate cyclase and function as auto- and heteroreceptors capable of reducing neuronal excitability and synaptic transmission (Conn and Pin 1997;Schoepp et al. 1999). Thus while group I mGluRs either enhance (Reid et al. 1999), or reduce the release of glutamate (White et al. 2003) via post-synaptic mechanisms (Pin and Duvoisin 1995), group II and III mGluRs decrease the release of glutamate (Pisani et al. 1997;Poisik O 2005).

mGluR2,4,7 and 8 are expressed on presynaptic neurons and mGluR3 and 5 on astrocytes indicating that most receptors are in the extrasynaptic domain. Both group II and III have been implicated in the modulation of glutamate neuronal transmission by presynaptic mechanisms (Battaglia et al. 1997).
mGluR2/3
mGluR2/3 receptors have been shown to influence a variety of glutamatergic dependent processes by either suppressing post-synaptic neuronal activity or inhibiting presynaptic release of glutamate (Nakanishi 1992). Generally group II mGluR are present presynaptically where they inhibit glutamate release (Loving and McCool 1995) and inhibited non-vesicular glutamate release from glia (Winder and Conn 1996). It has been hypothesized that one function of mGluR2/3 receptors is to regulate synaptic glutamate by providing negative feedback during periods of excessive glutamate release (Schoepp and Marek 2002). Furthermore they modulate glutamate transmission as autoreceptors located on glutamatergic terminals, or other neurotransmitters including GABA and dopamine as presynaptic heteroreceptors (Conn and Pin 1997;Cartmell et al. 2000;Zhao et al. 2001), and they can antagonise 5-hydroxytryptamine receptor 2A (5-HT2A) function (Marek et al. 2000).

mGlu 2/3 receptors are abundantly localized within forebrain regions including limbic structures (Ohishi et al. 1993a;Ohishi et al. 1993b). Among the limbic structures, the nucleus accumbens (NAc) in particular is being focused on with keen interest because of the central role of accumbal dopamine in the mediation of motivation and rewards (Ohishi et al. 1993a;Ohishi et al. 1993b). Microdialysis studies have shown that local application of a selective mGluR2/3 agonist and antagonist decreased and increased extracellular dopamine levels in the NAc respectively (Hu et al. 1999;Xi et al. 2002;Greenslade and Mitchell 2004).

Extensive pharmacological studies have indicated that mGlu2/3 receptors are associated with several neurological and psychiatric disorders (Swanson et al. 2005).
When this is taken into account, drugs which act on mGlu 2/3 receptors may hold promise for therapeutic benefit. Agonists of mGlu 2/3 receptors have shown to exhibited anxiolytic-like effects and antipsychotic-like activities in animal models (Swanson et al. 2005). For a review of mGlu 2/3 receptors see articles written by the following authors; (Conn and Pin 1997;Anwyl 1999;Schoepp 2001;Spooren et al. 2003).

6.3 Glia receptors
Numerous studies have demonstrated that all glial cell types express the same variety of neurotransmitter receptors as neurons (Verkhratsky & Kettenmann, 1996; Verkhratsky et al. 1998; Verkhratsky & Steinhauser, 2000). Many of these receptors, when activated by neural activity, initiate glial Ca\(^{2+}\) responses, which produce long-range interglial Ca\(^{2+}\) waves (Goldberg et al. 2010). Glial Ca\(^{2+}\) signals, in turn, activate release of
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gliotransmitters (which include glutamate, ATP, taurine, D-serine and probably many others) that can signal back to neurons, thus functionally integrating neuronal and glial circuitries (Bezzi et al. 1998, 2004; Zhang et al. 2004; Volterra & Meldolesi, 2005).

Gliial cells perform a multitude of functions, which to a great extent controls neuronal development, appearance, maintenance and plasticity of synaptic contacts. Further functions even include energy support, and integration into neuron–glia vascular components (Nedergaard et al. 2002; Verkhratsky, 2007). The most important however is the possibility that the astrocytes can carry out intercellular ‘volume’ transmission. This may allow them to play an unknown role in high cognitive functions.

Recent methodological advance in immunochemistry, molecular biology, and electrophysiology have brought new insight into glial-cell physiology and established that macroglial cell types, astrocytes and oligodendrocytes all express various types of glutamate receptors (Gallo 1995). Physiological, biochemical, and molecular studies show that both iGluR and mGluR are present on glial cells at a similar density to that found on neurons (Gallo 2000). This suggests that conventional neurotransmitters such as glutamate may have broader roles in cell signalling (Lin and Bergles 2004).

7. “Spillover” hypothesis of glutamate release

Based on the characteristics of glutamate synaptic transmission, a “spillover” hypothesis has been put forward to explain the nonproportional regulation of synaptic transmission. The first task of glutamate released in the synaptic cleft is to mediate rapid point to point transmission, but under certain conditions such as intense stimulus, the transmitter might also carry out more diffuse signals in perisynaptic environment. This escape of glutamate from the cleft provides a mechanism for activation of glutamate receptors that are located extrasynaptically, the most prominent of these being mGluR but also AMPA receptors in glia cells (Bergles et al. 1999). The extent of these phenomena depends on the amount and rate of escape, the distance between adjacent synapses, and the location and abundance of glutamate transporters (Bergles et al. 1999). Thus when glutamate escapes from the synaptic cleft (“spill-over”) it might transmit information to the extra- and heterosynaptic receptors before uptake takes place and therefore may also play a role in neuronal-glial communication.
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8. Compartmentalization of glutamate

The compartmentalization of glutamate is necessary to permit the various physiological functions. Various authors have discriminated between glutamate present in “neuronal” or “glial” pools. The glial pool is often referred to as “metabolic pool”.

Rothman showed in a study using $^{13}$C nuclear magnetic resonance spectroscopy measuring the flow of $^{13}$C label from glucose to glutamate to glutamine, that glutamate release and metabolic recycling cannot be conceptually distinguished from glutamate neurotransmission (Rothman et al. 2003). Therefore it is inaccurate to view metabolic and neurotransmitter glutamate from the neuronal pools as functionally distinct pools, but rather evidence of a highly coordinated interaction between neuronal and astrocytes needed to maintain glutamate signalling (Baker and Kalivas 2006).

The glutamate-glutamine cycle

The glutamate-glutamine cycle represents the conversion of glutamate in astrocytes by amidation to glutamine by the addition of ammonia. This reaction is catalyzed by the glial enzyme glutamine synthetase. Glutamine is then released into the extracellular space where it can be taken up into neurons and converted to glutamate by phosphatase-activated glutaminase. Therefore astrocytes do not return glutamate to neurons, but instead supply precursors that can be used for glutamate synthesis. Precursors that can be utilized by neurons to synthesize glutamate include glutamine as well as $\alpha$-ketoglutarate and tricarboxylic acid (TCA) cycle intermediates, including lactate or pyruvate (Sonnewald et al. 1997; Danbolt 2001).

Despite the importance of glutamate in both the synaptic and extrasynaptic space, surprisingly little is known regarding the relative importance of this cycle and its mechanisms to glutamate signalling, since both pools can stimulate glutamate receptors.
9. Glia cells

Glia cells comprise approximately half of the volume of the adult mammalian brain (Ventura and Harris 1999) and are the primary neuronal structural and trophic supportive elements. They were thought of as “glue” of the brain, which is based on the perceived function of these cells as structural cohesive elements. The glia cells are further defined as either macroglia (astrocytes, oligodendrocytes and NG2 glia) or microglia. Glia cells outnumber neurons in the mammalian CNS by about ten to one (Coyle and Schwarcz 2000). Compared to other primates, humans have an even higher glia to neuron ration in the frontal cortex, the area of the brain most associated with higher cognition.

Glia processes are not uniformly distributed in different brain regions; they are organized into distinct nonoverlapping domains and extent elaborate and dense fine processes that interact intimately with synapses and cerebrovasculature. The structural relationship
between astrocytes and synapses can change during development, in response to exogenous applied glutamate, and with altered neuronal functions. The location and distribution of astrocytic processes is important for regulating the extracellular milieu in the CNS.

In general astrocytes provide energy for neuronal function and modulate the formation and efficacy of synapses (Pfrieger and Barres 1996). The intracellular concentration of glutamate in neurons is in the order of 1-10 mM, whereas in astrocytes the concentration does not exceed 50 µM (due to the fact that glutamate in astrocytes is metabolized to glutamine) (Okumoto et al. 2005). Since glutamate does not easily penetrate the blood–brain barrier (McCall et al. 1979; Smith 2000) and neurons, unlike astrocytes, cannot synthesize glutamate from glucose, neurons are dependent on astrocytes. Therefore astrocytes remain crucial to maintain glutamate levels needed to support synaptic release.

In summary astrocytes regulate extracellular glutamate via glutamate transporters (Rothstein et al. 1994; Chaudhry et al. 1995) and recycle glutamate via glutamine and intermediates of the tricarboxylic acid cycle (Sonnewald et al. 1997). By clearing excess glutamate from the extracellular space and since glutamine does not stimulate glutamate receptors, astrocytes prevent excitotoxic glutamate concentrations that can lead to neuronal cell death (Porter and McCarthy 1997).

Nowadays it is evident that glia cells like astrocytes do not only serve as a simple “glue” of the brain but that they are able to communicate directly with neurons via cell-cell adhesion junctions (Spacek and Harris 1998), and intercellular calcium signalling (Parpura et al. 1994). The recognition in the mid 1990s that astrocytes undergo elevations in intracellular calcium concentrations following activation of G-protein coupled receptors by synaptically released neurotransmitters demonstrated not only that astrocytes display a form of excitability but also that astrocytes may be active participants in brain information processing. The roles that astrocytic calcium elevations play in neurophysiology and especially in the modulation of neuronal activity have been intensely researched in recent years (Agulhon et al. 2008).

10. Glutamate released from astroglia
Accumulated evidence indicate that glial cells are responsible for the release of a major part of the glutamate in the ECF. Astroglia have extensive projections and there is
accumulating evidence that at certain sites astroglia will release glutamate instead of taking it up.

Increasing evidence show that the astroglial network has the capacity to interfere actively with neurotransmission, e.g. it has recently been proposed that the degree of astroglial wrapping of the synapse might determine the distance that glutamate is able to migrate out of the synaptic cleft. Whether or not the glutamate plays a role at perisynaptic sites, the main portion of the transmitter will finally be taken up in the astroglial cell where the transmitter is rapidly converted to glutamine.

Glutamate can be released from certain sites of astroglia both during normal state as well as during pathological conditions. There are now several mechanisms of glutamate release from astroglia described. All these mechanism are calcium-independent. These mechanisms are described in Table 1 in Chapter 2, as well in the review from (Jabaudon et al. 1999;Cavelier et al. 2005;Xu et al. 2007;Malarkey and Parpura 2008)

11. Glutamate homeostasis
Extracellular glutamate in the brain is derived from both glia and synaptic release from neurons (Attwell et al. 1993). Recently, evidence has emerged that in addition to a metabolic relationship, nonsynaptic regulation of extracellular glutamate is important for maintaining the ability of glutamatergic synapses to undergo synaptic potentiation and depotentiation (Moussawi et al. 2009). This relationship between synaptic and nonsynaptic glutamate to regulate synaptic plasticity has been termed glutamate homeostasis (Kalivas 2009).

Essentially Kalivas et al. (2009) states that glutamate homeostasis involves the regulation of extracellular glutamate levels in the synaptic and perisynaptic extracellular environment. Affecting synaptic activity and plasticity by controlling glutamate access to ionotropic and metabotropic glutamate receptors. A crucial factor in maintaining glutamate homeostasis is the balance between glia and synaptic glutamate release and elimination. Well-developed cellular mechanisms exist to preserve glutamate homeostasis and regulate extrasynaptic glutamate levels. A mechanistic link between cystine-glutamate exchange and mGluR2/3 was shown in a study by (Moran et al. 2005). The cystine-exchange mediated elevation in extracellular glutamate, inhibited synaptic released glutamate by stimulating the mGluR2/3 presynaptic autoreceptors. The ability to quantify extracellular and synaptic concentrations of glutamate that provide tonus onto extrasynaptic metabotropic receptors responsible for regulating synaptic plasticity at
glutamatergic synapses is therefore critical to the concept of glutamate homeostasis (Moran et al. 2005).

Recent research indicates that disruptions in glutamate homeostasis is associated with addictive disorders. The disruptions in glutamate concentrations observed after prolonged exposure to drugs of abuse are associated with changes in the function and activity of several key components within the homeostatic control mechanism, including the cystine/glutamate exchanger and the glial glutamate transporter, EAAT2. Alterations in the balance between synaptic and extrasynaptic glutamate levels in turn influence signalling through presynaptic and postsynaptic glutamate receptors, and thus affect synaptic plasticity and circuit-level activity (Reissner and Kalivas 2010).

12. Other sources of extracellular glutamate
As blood levels of glutamate are found to be around 100 µmol/l, the general circulation should be considered as a possible source of extracellular glutamate. However studies that made use of blood infusion indicate that glutamate only penetrates the brain after a considerable delay.

Artificial sources of glutamate produced during microdialysis experiments should not be ruled out. It was recently shown that microbial contamination of the microdialysis system might confound glutamate detection. Sterilization of probes and tuning is necessary for implantation period longer than 24 h. In our laboratory we have observed that simply “handling” of microdialysis tubing can produce glutamate. These sources of glutamate are obviously not TTX dependent (Timmerman and Westerink 1997).

Recently the presence of D-glutamate in rat brain samples was reported. As D-glutamate is only transported by low affinity glutamate uptake systems and not converted inside astroglia to glutamine, its levels might accumulate in the ECF. As HPLC separation does not differentiate between the enantiomers, the possible contribution of D-glutamate to glutamate (the amino acid) levels in the ECF needs further investigation (Westerink et al. 2006).

13. Why study glutamate?
13.1 Glutamate synapses as therapeutic targets
There has been a great deal of interest in developing therapeutic strategies aimed to influence the glutamate synapse in a very wide range of neurological and psychiatric disorders. On the basis of multiple animal models and increasing human clinical data, it
is clear that the glutamate synapse is involved in stroke, amyotrophic lateral sclerosis, pain, epilepsy, amnesia, traumatic brain injury, Parkinson’s disease, addiction, depression, psychosis (such as schizophrenia), anxiety, Alzheimer’s, post-traumatic stress disorder and Huntington’s disease (Meldrum 1994).

Early clinical interest focused on limiting glutamate’s bad qualities. There are two general ways, that glutamate may damage nerve cells. First, there can be too much glutamate: abnormally high concentrations of glutamate can lead to over excitation of the receiving nerve cells. Second, the receptors for glutamate on the receiving neurons can become sensitized, actually to such an extend that less glutamate molecules are necessary to excite that cell. In both cases, cells activated by glutamate become overexcited. This over excitation can lead to an uncontrolled influx of $\text{Ca}^{2+}$ that causes cell damage and/or death. For this reason, glutamate is referred to as an excitotoxin when it causes cellular damage. Glutamate causes much of the damage that occurs after a stroke, and it’s also probably the chief neuron-killing villain in ALS and some other neurodegenerative diseases.

In general the ion channel receptor agonists and antagonists proved to be poor drug targets, because their effects are too nonselective, leading to dangerous side effects. Much attention was given to NMDA. For example NMDA antagonists can produce a variety of desirable effects, from blocking drug craving to mitigating the results of a stroke. However they cannot be used clinically because they induce e.g. psychotic symptoms. The hallucinogenic drug phencyclidine (PCP or ‘angel dust’), for example, is an NMDA antagonist.

**Addiction**

Researches are acknowledging the role that glutamate has in addiction. Although dopamine may fuel the “high” people feel from taking an addictive drug, it looks increasingly as though it’s glutamate that gets people addicted, as glutamate’s involvement in synaptic plasticity engraves new learning in the brain. The cough medicine Dextromethorphan, a weak NMDA antagonist, has shown to prevent tolerance to opioids in rats. Administration of an NMDA antagonist Dizocilpine (MK801) prevents rats from getting sensitized to cocaine.

**Anxiety disorder**

The role of the 5-HT and GABAergic system in anxiety disorders is well established. In addition recent data has shown that also glutamate neurons might be involved in these disorders. Preclinical data have implicated the NMDA receptor in the acquisition of
conditioned fear (Davis et al. 1994). Both stress and acute treatment of NMDAR antagonists increase prefrontal extracellular glutamate levels in rodents (Moghaddam 2002; Homayoun et al. 2005). Furthermore drugs that stimulate the metabotropic glutamate receptors mGlur2 and mGlur3 are supposed to decrease glutamate and have shown anxiolytic potential in preclinical models of fear and anxiety (Schoepp et al. 1999b). The group II agonist LY354740 has shown to be effective in reducing fear-of-shock induced startle potentiation and subjective anxiety in normal volunteers, although it was ineffective in potentiating darkness-induced startle augmentation.

**Schizophrenia**

Schizophrenia is another disorder that may be treated by glutamate targeting drugs. The glutamate theory of schizophrenia features a central role for the NMDA receptors. NMDA antagonists such as PCP and ketamine generate symptoms, including disturbed cognition and emotional withdrawal that bear an uncanny resemblance to those of the disease. mGlu receptor modulators are currently in an early stage of development for treatment of schizophrenia. The mGluR2/3 agonist LY2140023 was in a phase II clinical trail. Eli Lilly reported that this compound should be viewed for exploring the mGlu2/3 mechanism, but was not found adequate for phase III due to safety concerns.

**Depression**

Depression, the most common mental illness, may also yield to glutamate-based drugs. As opposed to schizophrenia where NMDAR agonists may prove effective, some data suggest a therapeutic role for NMDAR antagonists in depression (Javitt 2004). This feature was discovered in the early 1960s when tuberculosis patients unaccountably cheered up after treatment with an antibiotic, D-cycloserine, which is also an NMDA antagonists. Other NMDA antagonists such as amantadine, used to treat Parkinson’s disease and AD, have also shown antidepressant effects.

A growing body of preclinical research suggests that the NMDA glutamate receptors are implicated in the pathophysiology of major depression and the mechanism of action of antidepressants (Reus et al. 2010). In many studies NMDA receptor antagonists have been shown to be effective in animal models of depression and models that predict antidepressant activity. Future exploration of NMDA antagonists may prove potential benefits as antidepressant agents, but clinical applicability may be limited by the psychomimetic effects and the potential for misuse by many of theses agents. The NMDA receptor antagonists without psychomimetic properties in humans (e.g. memantine), however, merit further testing for antidepressant activity.
Interestingly in various clinical trials, the intravenous drug ketamine has been reported to produce an immediate antidepressant effect after a single dose, which persisted for over 72 hours following infusion in treatment-resistant major depressed patients (aan het Rot et al. 2010; Liebrenz et al. (2007); Matthews et al. 2010; Price et al. 2009; Zarate et al. 2006) and treatment-resistant bipolar depressed patients (Diazgranados et al. 2010).

Preclinical antidepressant-like effects have also been reported for group I metabotropic antagonists, which would be expected to inhibit NMDAR-mediated neurotransmission, as well as for group II and group III metabotropic agonist (Javitt 2004).

**Alzheimer's**
Alzheimer’s, shows widespread neuronal changes, indicating involvement of cortical glutamatergic systems. It has been hypothesized that glutamate induced excitotoxic effect may lead to widespread neuronal degeneration (Olney et al. 1997). NMDAR antagonists have been used to attempt to slow excitotoxic neurodegeneration. In particular, memantine, a weak NMDAR channel blocker, has shown safety and efficacy in slowing decline in moderate to advanced alzheimer’s. The tolerability of memantine relative to other channel blockers (PCP, MK801) appears to be due to its low affinity, fast unblocking kinetics and limited liability for trapping within closed channels.

**Post-traumatic stress disorder**
In post-traumatic stress disorder pathological associations are learned under conditions of extreme stress, and must subsequently be unlearned. NMDA receptors play a highly selective role in reversal learning. In animal models of post-traumatic stress disorder low doses of the partial NMDA agonist D-cycloserine stimulated reversal learning in septally and hippocampally lesioned rats.

**Possible other therapeutic targets**
In the case of AMPA receptors, positive allosteric modulators (AMPAkines) may be explored. Stimulating AMPA receptors increases levels of brain–derived growth factors. Possible applications are depression, Alzheimer’s and craving that drives addiction (Javitt 2004).

Other promising targets include regulator sites on the NMDA receptor. For example the allosteric site where the amino acid glycine binds; its stimulation facilitates NMDA receptor function and reduces the effects of ketamine. Studies have been conducted with
three separate agents: glycine and D-serine, which function as full agonists and D-
cycloserine, which functions as a partial agonist. High affinity antagonists for GLYT1-type
glycine transporters have also been developed and shown to be systemically active
(Javitt 2004).

Compounds that block specific subunits of NMDA receptors may provide new
opportunities too. Many NMDA receptors are made up of several subunits, the exact
combination of which may vary depending on the type of neuron on which the receptors
appear. A subunit of particular interest is called NR2B. The location or the areas where it
is concentrated, this receptor population looks like a promising target for both
Parkinson’s disease and pain control.

The discovery of mGluR in the early 90’s opened up a new world of potential drug
targets. However, targeting specific mGluRs proved very difficult. Eventually the
challenge of designing selective molecules led to modulation of allosteric sites of the
mGLU receptor that are distinct from the orthosteric binding site. Because they do not
bind on the active site of the mGlu receptor, they do not inhibit glutamate binding at all,
but either reduce or amplify the signal created by the endogenous ligand. The first
positive allosteric modulator (PAM) modulated mGluR1. This allosteric modulator was
followed by negative allosteric modulators (NAM) STX107 and AFQ056.

LY2140023 an mGluR2/3 orthosteric agonist is currently in a phase II trail has
encouraged others to pursue allosteric modulators of mGluR2/3. ADX63365 an mGluR5
PAM has shown activity in schizophrenia preclinical and AZD2066 a mGluR5 NAM is
currently in phase I. ADX10059 an mGluR5 NAM may be the furthest along mGluR
allosteric modulator in clinical development. It seems that the allosteric modulation sites
may represent a novel drug target.

Seeing the amount of clinical trails and research efforts, it is unfortunate that little
success in glutamate marketing has followed. The uncontrollable pharmacological nature
of the glutamate synapse leaves a window open for much needed future research.

13.2 A better understanding of the extracellular glutamate concentration
Although glutamate has a strong impact on brain physiology; the exact role of glutamate
basal levels in extrasynaptic cell-to-cell communication is still not clear. It has been
especially difficult to determine in conscious animals the origin and physiological
significance of extracellular glutamate. The glutamate concentrations as seen in the body
and brain are shown in Table 1. Affinities for the various subtypes of glutamate receptors are shown in Table 2, and the extracellular glutamate concentrations in the brain as measured with different methods are summarized in Table 3.

Glutamate concentrations in the extracellular space measured by means of microdialysis are estimated to be 1-4 µM (Lerma et al. 1986; Baker et al. 2002; Montiel et al. 2005; Nyitrai et al. 2006). Microsensor studies suggest even higher glutamate concentrations: between 1 – 50 µM. Given the efficacy of the glutamate uptake system it seems unrealistic that ambient glutamate concentrations in the synapses are constantly in this high range. Theoretically transporters are able to decrease the glutamate levels as low as 2 nM (Zerangue and Kavanaugh 1996; Levy et al. 1998b). In vitro hippocampal slices studies have shown data closer to this estimate. A concentration of 25 nM (Herman and Jahr 2007), 27-33 nM (Cavelier and Attwell 2005) and 83-87 nM (Le Meur et al. 2007) was detected by various researchers.

L-glutamate release in the mammalian brain is fast, on a millisecond scale, and the neuronal activity that release and metabolize L-glutamate is a spatially localized event. Therefore the development of a real-time monitoring method with high spatial and temporal resolution is crucial and the physical and chemical perturbations of target biological systems should be minimized. In Chapter 2 and 3 the development of glutamate microsensors as a method to measure glutamate was discussed and compared to the available method microdialysis. Where microdialysis literature indicated that glutamate was TTX and calcium independent, the first published glutamate sensoring experiments contradicted these notions (Kulagina et al. 1999; Oldenziel et al. 2006c). However experiments performed with the same and other types of glutamate microsensors (data not published) contradicted the findings of these initial articles. These findings are further discussed in Chapter 9.

A possible explanation for the reported high extracellular levels may be found in the fact that affinity transporter efficacy around the probe or sensor, are affected by the implantation. The latter assumption is supported by the relatively moderate effects of infusion (microdialysis) or injection (microsensor) of the high-affinity glutamate transport blocker TBOA (both methods show a 2-3 fold increase of glutamate concentrations).

However there is a second and more likely explanation for the relatively high values that both in vivo methods detect. Various authors have emphasized that glutamate might be functionally released from glial cells. In this regard several sources of non-synaptic
glutamate release have been identified. These mechanisms are discussed in further detail (Chapter 2.2.4.). As the glial cells exceed the neurons in number, it is not unlikely that microdialysis probes and microsensors mainly sample glutamate release and metabolism related to glial cells. Therefore unlike the other CNS transmitters, measurement of extracellular glutamate concentrations does not provide a useful index of synaptic release (Herrera-Marschitz et al. 1996). This could indicate that – at least the basal values – of glutamate concentrations detected by the present in vivo methods may not be directly translated to synaptic events.

| Table 1: Glutamate concentrations in the brain (Clements et al. 1992; Meldrum 2000; Danbolt 2001; Nedergaard et al. 2002; Featherstone and Shippy 2008) |
|---------------------------------|---------------------------------|
| Brain tissue (homogenate)       | 10 mmol/L (Meldrum et al. 2000) or 5-15 mmol/kg (Danbolt et al. 2001) |
| CSF                             | < 1 µmol/L (Meldrum et al. 2000) or 10 µmol/L (Danbolt et al. 2001) or 11.4 µmol/L (Lerma et al. 1986) |
| Brain Extracellular fluid (ECF) | 0.5-2 µmol/L (Meldrum et al. 2000) or 3-4 µmol/L (Danbolt et al. 2001) |
| Plasma                          | 30-100 µmol/L                  |
| Red Blood cell cytoplasmic glutamate | ± 500 µmol/L                |
| Synaptic cleft                  | 25 nmol/L (Herman and Jahr 2007) or 2-1000 µmol/L (Meldrum et al. 2000) |
| Synaptic vesicle                | 100 mmol/L                    |
| Neurons cytoplasm               | 5-10 mM                       |
| Astrocytes                      | 2-3 mM                        |
Table 2: Glutamate affinities in the brain (EC$_{50}$, ED$_{50}$, IC$_{50}$) (Patneau and Mayer 1990; Conn and Pin 1997; Meldrum 2000; Featherstone and Shippy 2008)

<table>
<thead>
<tr>
<th>Receptor Type</th>
<th>Range of Glutamate Affinity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLT-1</td>
<td>1–20 µmol/L</td>
</tr>
<tr>
<td>NMDA receptors</td>
<td>±1.3 µmol/L (Cavelier et al. 2005)</td>
</tr>
<tr>
<td>AMPA receptors</td>
<td>±4 µmol/L (Cavelier et al. 2005)</td>
</tr>
<tr>
<td>KA receptors</td>
<td>2.8–13 µmol/L (Cavelier et al. 2005)</td>
</tr>
<tr>
<td>mGluR1</td>
<td>11–56 µmol/L (Pin and Duvoisin 1995)</td>
</tr>
<tr>
<td>mGluR2</td>
<td>4–20 µmol/L (Pin and Duvoisin 1995; Conn and Pin 1997)</td>
</tr>
<tr>
<td>mGluR3</td>
<td>4–5 µmol/L (Pin and Duvoisin 1995; Conn and Pin 1997)</td>
</tr>
<tr>
<td>mGluR4</td>
<td>3–20 µmol/L (Conn and Pin 1997)</td>
</tr>
<tr>
<td>mGluR5</td>
<td>3–10 µmol/L (Pin and Duvoisin 1995; Conn and Pin 1997)</td>
</tr>
<tr>
<td>mGluR6</td>
<td>16 µmol/L (Pin and Duvoisin 1995; Conn and Pin 1997)</td>
</tr>
<tr>
<td>mGluR7</td>
<td>1000 µmol/L (Pin and Duvoisin 1995; Conn and Pin 1997)</td>
</tr>
<tr>
<td>mGluR8</td>
<td>0.02 µmol/L (Conn and Pin 1997)</td>
</tr>
</tbody>
</table>
Table 3: Extracellular glutamate concentration according to various authors

<table>
<thead>
<tr>
<th>Glutamate concentration</th>
<th>Area</th>
<th>Method</th>
<th>Author</th>
</tr>
</thead>
<tbody>
<tr>
<td>Theoretically</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>±2 nM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>±2 µM</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In vitro measured with Path clamp</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 nM</td>
<td>Acute slices</td>
<td>Patch clamp</td>
<td>(Herman and Jahr 2007)</td>
</tr>
<tr>
<td>27-98 nM</td>
<td>Acute slices</td>
<td>Patch clamp</td>
<td>(Cavelier and Attwell 2005)</td>
</tr>
<tr>
<td>83-87 nM</td>
<td>Acute slices</td>
<td>Patch clamp</td>
<td>(Le Meur et al. 2007)</td>
</tr>
<tr>
<td>In vitro measured with glutamate sensors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.7 ± 1.0 µM</td>
<td>Acute slices rat hippocampal (CA1)</td>
<td>Implanted Carbon fibre hydrogel coated E</td>
<td>(Oldenziel et al. 2006a; Oldenziel et al. 2007)</td>
</tr>
<tr>
<td>1.0 ± 0.1 to 10 ± 2 µM</td>
<td>Acute slices mouse hippocampal (CA1)</td>
<td>Implanted capillary E</td>
<td>(Oka et al. 2007)</td>
</tr>
<tr>
<td>10 ± 5 to 20 ± 12 µM</td>
<td>Acute slices mouse hippocampal (DG)</td>
<td>Implanted capillary E</td>
<td>(Oka et al. 2007)</td>
</tr>
<tr>
<td>30 ± 2 µM</td>
<td>Acute slices mouse hippocampal (DG)</td>
<td>Implanted capillary E</td>
<td>(Nakajima et al. 2003)</td>
</tr>
<tr>
<td>In vivo under anaesthesia measured with microdialysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.97 ± 0.7 µM</td>
<td>Anesthetized rat striatum</td>
<td>Push pull probe Microdialysis</td>
<td>(Kottegoda et al. 2002)</td>
</tr>
<tr>
<td>1.76 ± 0.15 µM</td>
<td>Anesthetized rat striatum</td>
<td>Fuse silica tubing combined with capillary electrophoresis</td>
<td>(Kennedy et al. 2002)</td>
</tr>
<tr>
<td>±1.3 -2.7 µM</td>
<td>Anesthetized rat striatum</td>
<td>Microdialysis</td>
<td>(Cellar et al. 2005)</td>
</tr>
<tr>
<td>±2 µM</td>
<td>Anesthetized rat hippocampus</td>
<td>Microdialysis</td>
<td>(Montiel et al. 2005)</td>
</tr>
<tr>
<td>2.9 ± 0.38 µM</td>
<td>Anesthetized rat hippocampus</td>
<td>Microdialysis</td>
<td>(Lerma et al. 1986)</td>
</tr>
<tr>
<td>In vivo in freely moving measured with microdialysis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2 ± 0.1 to 1.8 ± 0.1 µM</td>
<td>Freely moving mice striatum</td>
<td>Microdialysis</td>
<td>(Shakil et al. 2005)</td>
</tr>
<tr>
<td>2.75 ± 0.1 to 4.2 ± 0.1 µM</td>
<td>Freely moving mice striatum</td>
<td>Microdialysis</td>
<td>(Holmer et al. 2005)</td>
</tr>
<tr>
<td>1.33 ± 0.29 to 3.47 ± 0.67 µM (±2 µM)</td>
<td>Freely moving rat striatum</td>
<td>Microdialysis</td>
<td>(Baker et al. 2002)</td>
</tr>
<tr>
<td>0.24 ± 0.04 µM</td>
<td>Freely moving rat striatum</td>
<td>Microdialysis</td>
<td>(Di Cara et al. 2001)</td>
</tr>
<tr>
<td>0.48 ± 0.09 to 0.64 ± 0.18 µM</td>
<td>Freely moving rat striatum</td>
<td>Microdialysis</td>
<td>(Segovia et al. 2001)</td>
</tr>
<tr>
<td>1.3 ± 0.3 to 1.6 ± 0.2 µM</td>
<td>Freely moving rat striatum</td>
<td>Microdialysis</td>
<td>(Miele et al. 1996)</td>
</tr>
<tr>
<td>8.64 ± 0.5 µM</td>
<td>Freely moving rat PFC</td>
<td>Microdialysis</td>
<td>(Rocha et al. 1996)</td>
</tr>
<tr>
<td>±2 µM</td>
<td>Freely moving rat PFC</td>
<td>Microdialysis</td>
<td>(Boatell et al. 1995)</td>
</tr>
</tbody>
</table>
### In vivo under anaesthesia measured with glutamate sensors

<table>
<thead>
<tr>
<th>[μM]</th>
<th>Anesthetized rat</th>
<th>Region</th>
<th>Electrode Material</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0 ± 0.5</td>
<td>Chloral hydrate</td>
<td>Striatum</td>
<td>Pt E</td>
<td>(Rahman et al. 2005)</td>
</tr>
<tr>
<td>18.2 ± 9.3</td>
<td>Equitensine</td>
<td>Striatum</td>
<td>Carbon fibre hydrogel coated E</td>
<td>(Oldenziel et al. 2006b; Oldenziel et al. 2006c)</td>
</tr>
<tr>
<td>29.0 ± 9.0</td>
<td>Chloral hydrate</td>
<td>Striatum</td>
<td>Carbon fibre hydrogel coated E</td>
<td>(Kulagina et al. 1999)</td>
</tr>
<tr>
<td>1-5</td>
<td>Urethane</td>
<td>Dentate gyrus</td>
<td>Pt-Ir E</td>
<td>(Hu et al. 1994)</td>
</tr>
</tbody>
</table>

### In vivo under anaesthesia measured with micro electrode array (MEA)

<table>
<thead>
<tr>
<th>[μM]</th>
<th>Anesthetized rat</th>
<th>Region</th>
<th>Electrode Material</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.4 ± 0.2</td>
<td>Urethane</td>
<td>Striatum</td>
<td>MEA</td>
<td>(Day et al. 2006)</td>
</tr>
<tr>
<td>1.6 ± 0.3</td>
<td>Urethane</td>
<td>Frontal cortex</td>
<td>MEA</td>
<td>(Day et al. 2006)</td>
</tr>
<tr>
<td>18.9 ± 0.8 to 41.7 ± 1.2</td>
<td>Urethane</td>
<td>Striatum</td>
<td>MEA</td>
<td>(Nickell et al. 2005)</td>
</tr>
</tbody>
</table>

### In vivo freely moving measured with MEA

<table>
<thead>
<tr>
<th>[μM]</th>
<th>Anesthetized rat</th>
<th>Region</th>
<th>Electrode Material</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0 ± 1.2</td>
<td>Freely moving mice</td>
<td>Striatum</td>
<td>MEA</td>
<td>(Hascup et al. 2008)</td>
</tr>
<tr>
<td>3.3 ± 1.0</td>
<td>Freely moving mice</td>
<td>PFC</td>
<td>MEA</td>
<td>(Hascup et al. 2008)</td>
</tr>
<tr>
<td>7.3 ± 0.9</td>
<td>Freely moving rats</td>
<td>Striatum</td>
<td>MEA</td>
<td>(Rutherford et al. 2007)</td>
</tr>
<tr>
<td>44.9 ± 4.7</td>
<td>Freely moving rats</td>
<td>PFC</td>
<td>MEA</td>
<td>(Rutherford et al. 2007)</td>
</tr>
</tbody>
</table>