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Perspective

Glutamatergic neurotransmission in aging: a critical perspective

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Abstract

The effects of aging on glutamate neurotransmission in the brain is reviewed and evaluated. Glutamate is the neurotransmitter in most of the excitatory synapses and appears to be involved in functions such as motor behaviour, cognition and emotion, which alter with age. However, relatively few studies have been conducted to study the relationship between glutamate and aging of the brain. The studies presented here indicate the existence of a number of changes in the glutamatergic system during the normal process of aging. First, an age-related decrease of glutamate content in tissue from cerebral cortex and hippocampus has been reported, although it may be mainly a consequence of changes in metabolic activity rather than glutamatergic neurotransmission. On the other hand, studies in vitro and in vivo have shown no changes in glutamate release during aging. Since glutamate sampled in most of these studies is the result of a balance between release and uptake processes, the lack of changes in glutamate release may be due to compensatory changes in glutamate uptake. In fact, a reduced glutamate uptake capacity, as well as a loss in the number of high affinity glutamate transporters in glutamatergic terminals of aged rats, have been described. However, the most significant and consistent finding is the decrease in the density of glutamatergic NMDA receptors with age. A new perspective, in which glutamate interacts with other neurotransmitters to conform the substrates of specific circuits of the brain and its relevance to aging, is included in this review. In particular, studies from our laboratory suggest the existence of age-related changes in the interaction between glutamate and other neurotransmitters, e.g. dopamine and GABA, which are regionally specific.

Keywords: Brain aging; Glutamate; Neurotransmitters interaction

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1. Introduction

Aging could be defined as a process of progressive degeneration of an organism. Behaviourally, aged animals show impairments in cognitive (learning and memory), emotional (motivation) and motor functions (Jolles, 1986), alterations that are the consequence of changes that occur in the brain with age. Changes in aged brains include shrinkage and loss of neurons in several areas, such as the neocortex, hippocampus and substantia nigra (Coleman and Flood, 1987). Also, at the beginning of the aging process, dendritic growth (perhaps as a compensatory response to the degeneration of neighbouring neurons) has been described, followed by regression at older ages (Coleman and Flood, 1987). These morphological changes, accompanied by changes in neurotransmission, may explain the altered brain function that occurs during aging. Neurochemical studies have shown changes in cholinergic (Bartus et al., 1982), catecholaminergic (Roth et al., 1986; Morgan et al., 1987; Govoni et al., 1988) and peptidergic (Fliers and Swaab, 1986) neurotransmission with age. However, very few studies have investigated glutamate neurotransmission during aging. This review focuses on the effects of aging on glutamate content in brain tissue and glutamate neurotransmission in the brain, highlighting the age-related changes in glutamate interactions with other neurotransmitters.

2. Glutamate neurotransmission

Glutamate is the neurotransmitter that acts in most of the excitatory synapses in the mammalian central nervous system (CNS) (Fonnum, 1984; Orrego and Villanueva, 1993). Glutamatergic neurons are widely distributed through the CNS, mainly in the forebrain, where most of the cortical projections contain glutamate (Fagg and Foster, 1983; Cotman et al., 1987). In particular, the cortico-striatal and the cortico-cortical (hipoccampus, commisure) pathways have been extensively studied (Fagg and Foster, 1983; Peinado and Mora, 1986; Cotman et al., 1987) (Fig. 1).

The metabolism of glutamate in the brain is separated into two compartments: neuronal and glial (Fig. 2). The synthesis of glutamate occurs mainly from glutamine through the action of glutaminase, which is localized in the mitocondria of glutamatergic terminals (Fonnum, 1993). Regarding the catabolism of glutamate, the glial enzyme glutamine synthetase converts glutamate to glutamine (Norenberg and Martínez-Hernández, 1979; Fonnum, 1993). Thus, glutamate released from neurons is transported to glial cells where it is converted into glutamine, which, in turn, diffuses through the extracellular space into neurons to be used for the synthesis of glutamate.

Exocitotic calcium-dependent release of glutamate has been demonstrated in both in vitro and in vivo preparations (Fonnum, 1984; Nicholls, 1993; Sanchez-Prieto et al., 1994; Zilkha et al., 1995) (Fig. 2). Recent studies have shown that, as well as neurons, astrocytes can release glutamate and therefore, be actively involved in

glutamatergic neurotransmission (see review in Araque et al., 1999). Once released, glutamate may reach a very high concentration in the synaptic cleft (1–3 mM) (Clements, 1996), which allows the action of glutamate on postsynaptic receptors and, in certain circumstances, the spillover and diffusion of glutamate to act on extrasynaptic receptors (located perisynatically, in neighbouring synapses or in glial cells) (see review in Kullmann and Asztely, 1998) (Fig. 2). High-affinity glutamate transporters, located mainly in astrocyte processes surrounding glutamatergic terminals (Gegelashvili and Schousboe, 1998; Seal and Amara, 1999), seem to play a very important role in regulating the diffusion and therefore, the extrasynaptic actions of glutamate (Asztely et al., 1997).

These high-affinity transporters located in astrocytes are the main mediators in clearing of extracellular glutamate (Rothstein et al., 1996; Gegelashvili and Schousboe, 1998; Seal and Amara, 1999) (Fig. 2). The concentration of glutamate in the extracellular space of the brain is low (0.1–1 mM), which is not surprising because of the neurotoxic potential of glutamate (see e.g. Choi, 1988; Meldrum and Garthwaite, 1990); this neurotoxicity of glutamate, however, has been recently questioned (Obrenovitch and Urenjak, 1997). Five high affinity transporters of glutamate have been cloned which are dependent on sodium and potassium gradients (Gegelashvili and Schousboe, 1998; Seal and Amara, 1999). When these gradients are dissipated, the glutamate transporters could be reversed and the extracellular concentration of glutamate increased; it has been suggested that this calcium-independent release of glutamate may play a role in pathological circumstances, such as ischemia, although its involvement in the physiological release of glutamate is questioned (Nicholls, 1993; Attwell et al., 1993).

Two types of receptors mediate the actions of glutamate: ionotropic and metabotropic receptors (Sprengel and Seeburg, 1993; Hollmann and Heinemann, 1994; Ozawa et al., 1998) (Fig. 2). The ionotropic glutamate receptors can be distinguished by their pharmacological and electrophysiological properties: the *N*-

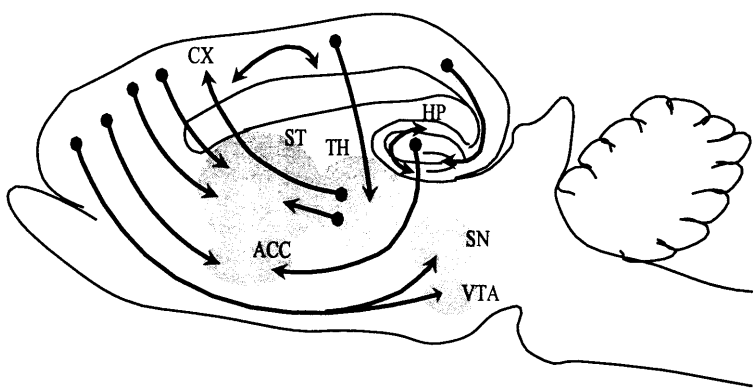

Fig. 1. Schematic diagram of the main glutamatergic pathways in the rat brain. ACC, nucleus accumbens; CX, cerebral cortex; HP, hippocampus; SN, substantia nigra; ST, striatum; TH, thalamus; VTA, ventro tegmental area. Modified from Cotman et al. (1987).

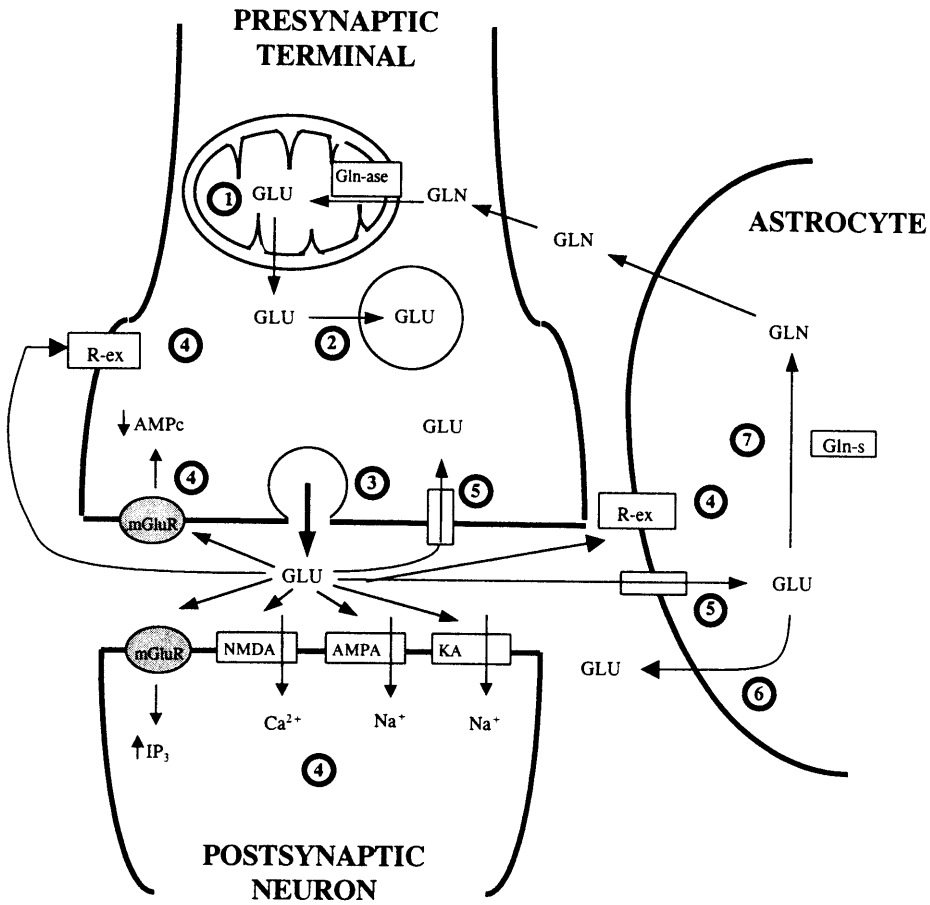

Fig. 2. Schematic representation of the processes related to the glutamate neurotransmission. (1) Synthesis of glutamate (GLU) from glutamine (GLN) through the action of glutaminase (Gln-ase). (2) Storing of glutamate into synaptic vesicles. (3) Exocitotic release of glutamate. (4) Glutamate activation of its receptors (AMPA, AMPA receptors; KA, kainate receptors; mGluR, metabotropic receptors; NMDA, NMDA receptors; R-ex, extrasynaptic receptors (ionotropic or metabotropic)). (5) Uptake of glutamate through high affinity transporters located in astrocytes and presynaptic terminals. (6) Synthesis of glutamine from glutamate through the action of glutamine sinthetase (Gln-s).

methyl-D-aspartate (NMDA) receptor, a channel highly permeable to Ca^{2+}, and the α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and the kainate receptor, which are Na^{+}-permeable channels. NMDA receptors are voltage-dependent channels and are highly regulated; they have been implicated in plasticity (learning and memory). AMPA receptors are the main mediators of the fast excitatory transmission by glutamate and their localization is similar to that of NMDA receptors. The role of kainate receptors is uncertain but they participate in the fast excitatory transmission. The metabotropic receptors exert their effects by

means of G-protein-initiated biochemical events. Eight subtypes of metabotropic receptors have been cloned, which mediate both excitatory and inhibitory actions of glutamate (Ozawa et al., 1998).

3. Aging of brain glutamatergic system

It has been suggested that glutamate is involved in a number of cerebral functions which become altered with age, such as learning and memory (see e.g. Collindridge and Bliss, 1987; Cotman et al., 1988), emotion and motivation (Mora and Cobo, 1990; Cobo and Mora, 1991) and motor functions (Schmidt et al., 1992). Glutamate seems to be involved in the pathogenesis of several age-related neurodegenerative disorders including Huntington's, Parkinson's and Alzheimer's diseases (see Choi, 1988; Greenamyre and Young, 1989; Meldrum and Garthwaite, 1990; Obrenovitch and Urenjak, 1997 for reviews). An increased susceptibility to glutamate induced toxicity with age has also been described (Liu et al., 1996; Brewer, 1998), which may be due in part to a reduction in the concentration of antioxidants (Vatassery et al., 1998; Lynch, 1998).

Studies on the aging of the brain are complicated by several confounding factors. The most evident are those related to species, strains and individual variability of the animals. For example, several reports have shown an age-related loss of neurons in various cerebral areas in human and monkey, but not in the rodent (Coleman and Flood, 1987). Also, differences between strains in how a particular parameter, such as NMDA receptor density, undergoes a change with age have been described (Petersen and Cotman, 1989). This variability is mainly due to the fact that chronological age is not the best indicator of the aging process and no biological markers are available to determine the onset of aging. So, in this review, to avoid interspecies variability and since research on brain aging has been carried out mainly in rodents, the results reported in the scientific literature obtained with rodent brains are considered in the light of the limitations mentioned above.

Two methodological factors should be underlined. First, erroneous conclusions could be drawn from studies that examined only two groups of age; studies on aging should be performed using animals of several ages (Coleman and Flood, 1987). This recommendation circumvents the problem of differentiating maturation and aging processes. Secondly, the brain is a heterogeneous organ with anatomically and physiologically different areas which may be affected in different manners by the aging process. Studies examining the whole brain or large cerebral regions, which include a number of heterogeneous areas, can complicate the interpretation of data. All these factors are taken into account in the present review.

3.1. Brain glutamate content

Initial studies of the effects of aging on glutamatergic neurotransmission investigated the content of glutamate in cerebral tissue samples from both origin and destination areas of the glutamatergic pathways. Table 1 summarizes available data for changes in glutamate content in brain tissue during aging.

Several reports have shown a decrease in glutamate concentration in tissue samples taken from the whole cerebral cortex of rodents as a result of age (Davis and Himwich, 1975; Strolin-Benedetti et al., 1990, 1991; Saransaari and Oja, 1995). The cerebral cortex is a heterogeneous structure and age-related changes in glutamate content may occur at different rates in different areas. In fact, studies performed in tissue samples of discrete areas of the cerebral cortex of Fischer-344 and Wistar rats show significant decreases in glutamate concentration in the frontal region, by 15–20% at 21–24 months (Fornieles et al., 1986; Dawson et al., 1989; Wallace and Dawson, 1990) but not in other areas, such as the parietal and temporal cortices (Fornieles et al., 1986; Banay-Schwartz et al., 1989). It is interesting that differences seem to exist between frontal areas. For instance, an age-related decrease in glutamate has been reported in the medial prefrontal cortex but not in the sulcal or dorsal prefrontal cortex (Fornieles et al., 1986). In contrast, no significant changes in frontal glutamate content have been reported in other rat strains (e.g. Long–Evans rats) in this same area of the brain (Dawson and Wallace, 1992). Since Long–Evans rats seem to have a longer life span (Coleman and Flood, 1987), it is possible that decreases could be found in glutamate in the frontal cortex of this rat strain at older ages.

In the hippocampus of mice, Fisher-344 and Wistar rats, the glutamate content in tissue samples seems to decrease with age (Banay-Schwartz et al., 1989; Strolin-Benedetti et al., 1990, 1991; Saransaari and Oja, 1995). It is interesting that these decreases in glutamate content have been consistently found at 12, 18, 21–24 and 29 months, reaching 6–8% in 21 months rats (Strolin-Benedetti et al., 1990, 1991) and 23% in 29 months rats (Banay-Schwartz et al., 1989), whereas no changes in glutamate with age have been reported in the hippocampus of Long–Evans rats (Dawson and Wallace, 1992).

The decrease in glutamate content in the frontal cortex is in agreement with the neuronal loss described in this area of the brain (Mufson and Stein, 1980) as well as in the cerebral cortex of aged rodents (Knox, 1982; Heumann and Leuba, 1983; Peters et al., 1987). The decrease in the glutamate content of the hippocampus also agrees well with the age-related decrease in neuronal density reported in the CA1 and CA3 areas (Brizzee and Ordy, 1979; Landfield et al., 1981). Since glutamate is the neurotransmitter of most cortical and hippocampal neurons (Fonnum, 1984), the glutamate decrease in tissue samples from these structures could be interpreted as a reflection of neuronal loss. Metabolic deficits in biochemical pathways that generate or utilize glutamate as a substrate could also contribute to these decreases. For example, changes in the regulation of glutaminase, the enzyme responsible for the hydrolysis of glutamine to form glutamate and ammonia, have been described in certain areas of the aged brain (Wallace and Dawson, 1992, 1993).

In rodent striatum, controversial results have been reported: a decrease of 15% (Strolin-Benedetti et al., 1990, 1991), no changes (Wallace and Dawson, 1990; Dawson and Wallace, 1992; Saransaari and Oja, 1995) and even increases (Donzanti and Ung, 1990) in glutamate content with age. Since the striatum is a heterogeneous structure, these results could be related to the anatomical and neurochemical differences within this structure. In fact, in aged rats, Donzanti and

Table 1
Changes in glutamate content in brain tissue during aging

Brain area	Animals	Age (months)	Changes	Units	References
Whole cerebral cortex	Wistar rats	3 vs. 21–22	↓9%, $P<0.05$	μmoles/g wet tissue	(Strolin-Benedetti et al., 1990)
Whole cerebral cortex	Wistar rats	3 vs. 21–22	↓10%, $P<0.05$	μmoles/g wet tissue	(Strolin-Benedetti et al., 1991)
Whole cerebral cortex	NMRI mice	3, 6, 12, 18, 24	↓ ≈ 30% at 12 months ↓ ≈ 30% at 18 months ↓ ≈ 45% at 24 months, $P<0.01$	mmoles/kg protein	(Saransaari and Oja, 1995)
Frontal cortex	Fischer-344 rats	6 vs. 24	↓16%, $P<0.01$	μmoles/g wet tissue	(Dawson et al., 1989)
Frontal cortex	Fischer-344 rats	6 vs. 24	↓18%, $P<0.05$	μmoles/g wet tissue	(Wallace and Dawson, 1990)
Medial prefrontal cortex	Fischer-344 rats	6 vs. 20	Not significant	ng/μg protein	(Donzanti and Ung, 1990)
Medial prefrontal cortex	Wistar rats	2, 12, 21	↓9% at 12 months, $P<0.05$	μmoles/g wet tissue ↓17% at 21 months, $P<0.05$	(Fornieles et al., 1986)
Dorsal prefrontal cortex	Wistar rats	2, 12, 21	Not significant	μmoles/g wet tissue	(Fornieles et al., 1986)
Sulcal prefrontal cortex	Wistar rats	2, 12, 21	Not significant	μmoles/g wet tissue	(Fornieles et al., 1986)
Temporal cortex	Wistar rats	2, 12, 21	Not significant	μmoles/g wet tissue	(Fornieles et al., 1986)
Occipital cortex	Fischer-344 rats	3 vs. 29	Not significant	nmoles/mg protein	(Banay-Schwartz et al., 1989)
Hippocampus	Wistar rats	3 vs. 21–22	↓6%, $P<0.05$	μmoles/g wet tissue	(Strolin-Benedetti et al., 1990)
Hippocampus	Wistar rats	3 vs. 21–22	↓8%, $P<0.05$	μmoles/g wet tissue	(Strolin-Benedetti et al., 1991)
Hippocampus	NMRI mice	3, 6, 12, 18, 24	↓ ≈ 20% at 12 months ↓ ≈ 30% at 18 months ↓ ≈ 15% at 24 months	μmoles/kg protein	(Saransaari and Oja, 1995)
Hippocampus	Fischer-344 rats	3 vs. 29	↓23%, $P<0.02$	nmoles/mg protein	(Banay-Schwartz et al., 1989)
Hippocampus	Fischer-344 rats	6 vs. 24	Not significant	μmoles/g wet tissue	(Wallace and Dawson, 1990)
Striatum	Sprague–Dawley rats	3, 4, 6, 10, 19	↓17–20%, $P<0.001$	nmoles/mg protein	(Price et al., 1981)

Table 1 (*Continued*)

Brain area	Animals	Age (months)	Changes	Units	References
Striatum	Wistar rats	3 vs. 21–22	↓14%, $P<0.01$	μmoles/g wet tissue	(Strolin-Benedetti et al., 1990)
Striatum	Wistar rats	3 vs. 21–22	↓15%, $P<0.01$	μmoles/g wet tissue	(Strolin-Benedetti et al., 1991)
Striatum	NMRI mice	3, 6, 12, 18, 24	Not significant	mmoles/kg protein	(Saransaari and Oja, 1995)
Striatum	Fischer-344 rats	6 vs. 21	Not significant	μmoles/g wet tissue	(Wallace and Dawson, 1990)
Striatum anterior dorso medial	Fischer-344 rats	6 vs. 20	Not significant	ng/μg protein	(Donzanti and Ung, 1990)
Striatum anterior dorso lateral	Fischer-344 rats	6 vs. 20	↑40%, $P<0.05$	ng/μg protein	(Donzanti and Ung, 1990)
Striatum anterior ventro lateral	Fischer-344 rats	6 vs. 20	↑21%, $P<0.05$	ng/μg protein	(Donzanti and Ung, 1990)
Striatum medial dorso medial	Fischer-344 rats	6 vs. 20	Not significant	ng/μg protein	(Donzanti and Ung, 1990)
Striatum medial dorso lateral	Fischer-344 rats	6 vs. 20	↑35%, $P<0.05$	ng/μg protein	(Donzanti and Ung, 1990)
Striatum medial ventro lateral	Fischer-344 rats	6 vs. 20	↑24%, $P<0.05$	ng/μg protein	(Donzanti and Ung, 1990)
Striatum posterior dorso medial	Fischer-344 rats	6 vs. 20	Not significant	ng/μg protein	(Donzanti and Ung, 1990)
Striatum posterior ventro lateral	Fischer-344 rats	6 vs. 20	↑18%, $P<0.05$	ng/μg protein	(Donzanti and Ung, 1990)

Ung reported increases of 20–40% in the glutamate content in several striatal subregions (dorsolateral and ventrolateral regions), but not in others (dorsomedial regions) (Donzanti and Ung, 1990).

Few studies have investigated the effects of age on glutamate content in other subcortical structures. In the nucleus accumbens, both decreases and no changes have been described (Donzanti and Ung, 1990; Strolin-Benedetti et al., 1990, 1991). In the substantia nigra, decreases (Strolin-Benedetti et al., 1990, 1991), no changes (Banay-Schwartz et al., 1989) and increases (Donzanti and Ung, 1990) in glutamate content have been reported.

In brief, the most consistent finding on the effects of aging on glutamate content in tissue from different brain areas is a decrease in prefrontal cortex and in hippocampus. It has been suggested that these data are related to the cognitive and memory deficits reported to occur with age and attributed, at least in part, to functions of the prefrontal cortex and hippocampus. However, since 70–80% of tissue glutamate is present in the metabolic pool and only 20–30% in glutamatergic nerve terminals (Fonnum, 1993), decreases in glutamate content may be mainly a consequence of a change in metabolic activity. Whether or not these decreases in glutamate content in prefrontal cortex and hippocampus are involved in behavioural deficits remains to be elucidated.

To investigate whether the effects of age on glutamatergic neurotransmission occur in a given area of the brain, it is necessary to study both presynaptic and postsynaptic markers, such as release, number of high-affinity uptake sites, transport or receptor affinity, receptor density or postsynaptic effects, in cerebral structures containing glutamatergic terminals. Studies of the effects of age on these glutamatergic markers are reviewed below.

3.2. Glutamate release

Neurotransmitter release can be investigated using both in vitro and in vivo techniques. The in vitro studies utilize brain slices or isolated nerve terminals (synaptosomes) and analyze the content of glutamate in the superfusion medium. These techniques provide the simplest model to investigate neurotransmitter release (Nicholls, 1993; Sanchez-Prieto et al., 1994). In contrast, in vivo studies using push–pull or microdialysis techniques, analyze the concentration of glutamate in samples obtained from the extracellular space of a specific area of the brain (Benveniste, 1989; Westerink and Justice, 1991; Porras and Mora, 1995; Segovia et al., 1997). Data on changes in basal and induced glutamate release during aging are summarized in Tables 2 and 3, respectively.

In the cerebral cortex, most of the in vitro and in vivo studies have shown no significant changes in basal extracellular concentrations of glutamate during aging. This remarkable stability of glutamate concentration seems to occur independently of the areas (frontal, parietal and occipital cortices), species (rat and mouse) and strains (Wistar, Long–Evans and Fischer-344 rats) (Dawson et al., 1989; Mora and Cobo, 1991; Cobo et al., 1992 Dawson and Wallace, 1992; Palmer et al., 1994; Saransaari and Oja, 1995; Porras et al., 1997). Also, the release of glutamate in the

Table 2
Changes in basal glutamate release during aging

Brain area	Animals	Age (months)	Changes	Technique	References
Whole cerebral cortex	Fisher-344 rats	6 vs. 28	Not significant	In vitro (slices)	(Dawson and Wallace, 1992)
Whole cerebral cortex	NMRI mice	3, 6, 12, 18, 24	Not significant	In vitro (slices)	(Saransaari and Oja, 1994)
Whole cerebral cortex	NMRI mice	3 vs. 24	Not significant	In vitro (slices)	(Saransaari and Oja, 1995)
Neocortex	Hybrid rats	3, 12, 24, 37	Not significant	In vitro (slices)	(Palmer et al., 1994)
Frontal cortex	Fischer-344 rats	6 vs. 24	Not significant	In vitro (slices)	(Dawson et al., 1989)
Sulcal prefrontal cortex	Wistar rats	3–4 vs. 24–26	Not significant	In vivo (push–pull)	(Cobo et al., 1992)
Medial prefrontal cortex	Wistar rats	3–4 vs. 24–26	Not significant	In vivo (push–pull)	(Cobo et al., 1992)
Medial prefrontal cortex	Wistar rats	3–4 vs. 28–30	Not significant	In vivo (push–pull)	(Cobo et al., 1993)
Medial prefrontal cortex	Wistar rats	2–3, 11–14, 24–26	Not significant	In vivo (push–pull)	(Porras et al., 1997)
Parieto-temporal cortex	Wistar rats	3–4 vs. 24–26	Not significant	In vivo (push–pull)	(Cobo et al., 1992)
Temporal cortex	Fischer-344 rats	8 vs. 28–30	Not significant	In vitro (slices)	(Meldrum et al., 1992)
Occipital cortex	Wistar rats	3–4 vs. 24–26	Not significant	In vivo (push–pull)	(Cobo et al., 1992)
Hippocampus	Balb/c mice	3 vs. 30	↑94%, $P<0.05$	In vitro (slices)	(Freeman and Gibson, 1987)
Hippocampus	NMRI mice	3 vs. 24	↓ ≈ 60%	In vitro (slices)	(Saransaari and Oja, 1995)
Hippocampus	Fischer-344 rats	8 vs. 28–30	Not significant	In vitro (slices)	(Meldrum et al., 1992)
Hippocampus	Wistar rats	3 vs. 22–24	↑ ≈ 68%, $P<0.05$	In vivo (microdialysis)	(Massieu and Tapia, 1997)
Striatum	Balb/c mice	3 vs. 30	↑77%, $P<0.05$	In vitro (slices)	(Freeman and Gibson, 1987)
Striatum	NMRI mice	3 vs. 24	Not significant	In vitro (slices)	(Saransaari and Oja, 1995)
Striatum	Wistar rats	2–3, 12–13, 24–34	Not significant	In vivo (push–pull)	(Porras and Mora, 1995)
Striatum	Wistar rats	3, 12, 22	↓40%, $P<0.05$	In vivo (microdialysis)	(Corsi et al., 1997)
Striatum	Wistar rats	3 vs. 22–24	↑ ≈ 190%, $P<0.05$	In vivo (microdialysis)	(Massieu and Tapia, 1997)
Striatum	Wistar rats	3 vs. 22	Not significant	In vivo (microdialysis)	(Corsi et al., 1999)
Striatum	Wistar rats	2–3, 12–14, 27–32, 37	Not significant	In vivo (microdialysis)	(Segovia et al., 1999a)
Lateral striatum	Fischer-344 rats	4, 12, 18, 24–26	↑	In vivo (microdialysis)	(Donzanti et al., 1993)
Medial striatum	Fischer-344 rats	4, 12, 18, 24–26	Not significant	In vivo (microdialysis)	(Donzanti et al., 1993)
Nucleus accumbens	Wistar rats	2–3, 12–14, 27–32, 37	Not significant	In vivo (microdialysis)	(Segovia et al., 1999a)

Table 3
Changes in induced glutamate release during aging

Brain area	Animals	Age (months)	Changes	Technique	Stimulation	References
Whole cerebral cortex	NMRI mice	3 vs. 24	$\uparrow \approx 50\%$	In vitro (slices)	K^+ 50 mM	(Saransaari and Oja, 1995)
Whole cerebral cortex	Wistar rats	3 vs. 27–30	Not significant	In vitro (synaptosomes)	4-aminopyridine 1 mM	(Sanchez-Prieto et al., 1994)
Neocortex	Hybrid rats	3, 12–24, 37	Not significant	In vitro (slices)	K^+ 50 mM	(Palmer et al., 1994)
Frontal cortex	Fischer-344 rats	6 vs. 24	Not significant	In vitro (slices)	K^+ 56 mM	(Dawson et al., 1989)
Medial prefrontal cortex	Wistar rats	3–4 vs. 28–30	$\downarrow \approx 50\%$, $P<0.05$	In vivo (push–pull)	Electrical	(Cobo et al., 1993)
Temporal cortex	Fischer-344 rats	8 vs. 28–30	$\uparrow \approx 2$-fold, $P<0.05$	In vitro (slices)	Electrical	(Meldrum et al., 1992)
Hippocampus	Balb/c mice	3 vs. 30	$\downarrow \approx 80\%$	In vitro (slices)	K^+	(Freeman and Gibson, 1987)
Hippocampus	NMRI mice	3 vs. 24	$\uparrow \approx 5$-fold, $P<0.01$	In vitro (slices)	K^+ 50 mM	(Saransaari and Oja, 1995)
Hippocampus	Fischer-344 rats	8 vs. 28–30	$\uparrow \approx 2$-fold, $P<0.05$	In vitro (slices)	Electrical	(Meldrum et al., 1992)
Striatum	Balb/c mice	3 vs. 30	$\downarrow 66\%$, $P<0.05$	In vitro (slices)	K^+	(Freeman and Gibson, 1987)
Striatum	NMRI mice	3, 6, 12, 18, 24	$\downarrow \approx 30\%$, $P<0.01$	In vitro (slices)	K^+ 50 mM	(Saransaari and Oja, 1995)
Striatum	Wistar rats	3 vs. 27–30	Not significant	In vitro (synaptosomes)	4-Aminopyridine 1 mM	(Sanchez-Prieto et al., 1994)
Striatum	Wistar rats	3, 12, 22	Not significant	In vivo (microdialysis)	K^+ 100 mM	(Corsi et al., 1997, 1999)

whole cerebral cortex induced by depolarizing agents, such as K^+ or 4-aminopyridine, seems to be unchanged with age (Dawson et al., 1989; Palmer et al., 1994; Sanchez-Prieto et al., 1994). Only one study in vitro has reported an increase in the release of glutamate induced by K^+ up to 50% in aged versus young mice (Saransaari and Oja, 1995).

As shown above, extracellular levels of glutamate did not change in the frontal cortex during aging despite neuronal loss, which suggests that functional compensations are made by surviving neurons (Dawson et al., 1989; Mora and Cobo, 1991; Cobo et al., 1992; Porras et al., 1997). However, a study from our laboratory showed that electrical stimulation at intensities that release glutamate in young rats does not increase glutamate in aged rats although the basal levels were similar in both age groups (Cobo et al., 1993). This change could be due to a shift in the excitability of the prefrontal cortex in old animals that could make the stimulation intensity use a sub-threshold for influencing release. This is likely, since an increase in the release of glutamate in the prefrontal cortex of aged rats was obtained with an increase in the intensity of electrical stimulation. In any case, the study of Cobo et al. suggests that neuronal changes, such as a decrease in excitability, do occur with age in the prefrontal cortex of the rat (Cobo et al., 1993).

In the hippocampus, basal extracellular concentrations of glutamate in aged rodents have been reported to be 94% greater (Freeman and Gibson, 1987), equal (Meldrum et al., 1992) or 60% lower (Saransaari and Oja, 1995) compared with those of adult rodents in in vitro studies. An in vivo study has reported an increase of basal glutamate release in 24-month-old as compared with 3-month-old Wistar rats (Massieu and Tapia, 1997). In potassium induced release of glutamate in aged animals there are reports of no change (Freeman and Gibson, 1987) and of increases by more than double (Meldrum et al., 1992; Saransaari and Oja, 1995).

In striatum, in vitro and in vivo studies have shown no changes in basal (Porras and Mora, 1995; Saransaari and Oja, 1995; Corsi et al., 1999; Segovia et al., 1999a) or chemically induced (Donzanti et al., 1993; Sanchez-Prieto et al., 1994; Corsi et al., 1997, 1999) extracellular concentrations of glutamate in aged rats. In contrast, there have been reports of both increases and decreases in the basal release of glutamate in the striatum (Freeman and Gibson, 1987; Massieu and Tapia, 1997; Corsi et al., 1997) and also a decrease in potassium induced relase of glutamate (Freeman and Gibson, 1987; Saransaari and Oja, 1995). Interestingly, one study described regional differences in age-related changes in basal extracellular concentrations of glutamate in striatum: an age-related increase in the extracellular concentration of glutamate in the lateral, but not the medial striatum of rats (Donzanti et al., 1993). It is possible that an actual increase in glutamate concentrations in the lateral striatum could be hidden in the studies of the whole striatum. These considerations are reinforced by the recent data of dopamine and GABA obtained in the striatum using microdialysis, in which a clear regional difference between dorsal and ventral striatum was found (Segovia et al., 1999a; Segovia, 1999).

To sum up, it might be said from the data revised above — in particular data derived from synaptosomes studies — that no changes in glutamate release seem to

occur during aging in cerebral cortex and striatum. However, the concentration of glutamate sampled in most of these studies (slices, push–pull, microdialysis) is the result of a balance between the release and uptake processes, so age-related decreases in presynaptic release of glutamate may be compensated by changes in uptake. On the other hand, push–pull and microdialysis techniques cannot differentiate between neuronal or glial sources of glutamate, so the cellular origin of the glutamate released is also uncertain (Timmerman and Westerink, 1997). Thus, decreases in glutamate release from the neuronal compartment could be masked by increases of glutamate release from non-neuronal sources.

3.3. Glutamate uptake

The main mechanism for clearing extracellular glutamate is the action of high-affinity transporters located mainly in astrocyte processes surrounding glutamatergic terminals (Rothstein et al., 1996; Gegelashvili and Schousboe, 1998). Table 4 summarizes data on changes in glutamate uptake during aging.

In the cerebral cortex and striatum, studies show a reduced ability to take up glutamate by aged rats (Wheeler and Ondo, 1986; Najerahim et al., 1990; Saransaari and Oja, 1995; Vatassery et al., 1998). The magnitude of this decrease in glutamate uptake with age has been averaged as 20–30% in rats at 24 months of age. In mice, this decrease has been estimated at 70–80%. In agreement with these findings, changes in the modulation by protein kinase C of the uptake of glutamate have been also reported (Daniels and Vickroy, 1998). In contrast, other studies have reported increases (Strong et al., 1984) or no changes (Dawson et al., 1989; Palmer et al., 1994) in glutamate uptake.

Several reports indicate a decrease in the maximal velocity of glutamate uptake (Price et al., 1981; Wheeler and Ondo, 1986). This could be interpreted as a loss in the number of high-affinity glutamate transport sites in these brain areas. The hippocampus exhibits a consistently unaltered uptake of glutamate during aging (Gilad et al., 1990; Najerahim et al., 1990; Palmer et al., 1994).

As shown above, very few studies have addressed the effects of aging on glutamate uptake, but most reported a lower uptake capacity for glutamate as well as a loss in the number of high affinity glutamate transport sites in the glutamatergic terminals of aged rats in both striatum and prefrontal cortex (Price et al., 1981; Wheeler and Ondo, 1986; Wheeler and Ondo, 1991; Najerahim et al., 1990; Vatassery et al., 1998; Saransaari and Oja, 1995). This conclusion should be drawn with caution when referred to its functional significance. That is, a decrease in the number of transport sites in nerve terminals could be compensated by increases in the affinity of the remaining sites (Price et al., 1981; Saransaari and Oja, 1995). Moreover, glutamate is taken up mainly into glial cells (Rothstein et al., 1996; Gegelashvili and Schousboe, 1998) and age-related changes in the uptake of glutamate seem to occur mainly in neurons (Daniels and Vickroy, 1998). Since astrocytes seem to increase in number and/or activity in the aging brain (Brizzee et al., 1983; Terry, 1986; Vazquez et al., 1992; David et al., 1997), this could compensate for decreases in neuronal glutamate uptake. In fact, one study using

Table 4
Changes in glutamate uptake during aging

Brain area	Animals	Age (months)	Changes in velocity	Changes in affinity	Technique	References
Forebrain	Hybrid rats	5 vs. 37	↓21% at 37 months		Synaptosomes	(Daniels and Vickroy, 1998)
Whole cerebral cortex	Long–Evans rats	2, 10, 18, 30	↓13% at 10 months ↓21% at 18 months ↓21% at 30 months	↑6–11%	Synaptosomes	(Wheeler and Ondo, 1986)
Whole cerebral cortex	NMRI mice	3, 6, 12, 18, 24	↓83% at 24 months, $P<0.01$	↑41% at 18 months, $P<0.01$ ↑71% at 24 months, $P<0.01$	Synaptosomes	(Saransaari and Oja, 1995)
Neocortex	Wistar rats	4, 12, 24	↓22%, $P<0.05$		Tissue homogenate	(Najerahim et al., 1990
Neocortex	Hybrid rats	3, 12, 24, 37	Not significant		Slices	(Palmer et al., 1994)
Frontal cortex	Fischer-344 rats	6 vs. 24	Not significant		Slices	(Dawson et al., 1989)
Hippocampus	Wistar rats	4, 12, 24	Not significant		Tissue homogenate	(Najerahim et al., 1990)
Hippocampus	Wistar–Kyoto rats Brown-Norway rats	3 vs. 20	Not significant		Synaptosomes	(Gilad et al., 1990)
Hippocampus	Hybrid rats	3, 12, 24, 37	Not significant		Slices	(Palmer et al., 1994)
Striatum	Sprague –Dawley rats	3, 4, 6, 10, 19	↓41% at 10 mo	↑32% at 10 months	Synaptosomes	(Price et al., 1981)

Table 4 (*Continued*)

Brain area	Animals	Age (months)	Changes in velocity	Changes in affinity	Technique	References
Striatum	Wistar rats	4, 12, 24	↓46% at 19 mo ↓29% at 12 mo Not significant at 24 months	↑36% at 19 months	Tissue homogenate	(Najerahim et al., 1990)
Striatum	Hybrid rats	3, 12, 24, 37	Not significant		Slices	(Palmer et al., 1994)
Striatum, rostral	Sprague–Dawley rats	7, 17, 27	↑84% at 17 months, $P<0.05$ ↑79% at 27 months, $P<0.05$	↓116% at 17 months, $P<0.05$ ↓102% at 27 months, $P<0.05$	Synaptosomes	(Strong et al., 1984)
Striatum, caudal	Sprague–Dawley rats	7, 27	Not significant	↓105% at 17 months, $P<0.05$ Not significant at 27 months	Synaptosomes	(Strong et al., 1984)

cortical slices, which include glial elements, reported no change in glutamate uptake with age (Dawson et al., 1989).

3.4. Glutamate receptors

Two types of receptors mediate the depolarizing action of glutamate: ionotropic and metabotropic receptors (Sprengel and Seeburg, 1993; Hollmann and Heinemann, 1994). Table 5 gives data on changes in glutamate receptors during aging.

A decrease with age in the density of glutamatergic receptors of the NMDA type has been reported. This is probably one of the most consistent findings in relation to glutamate and aging of the brain. Decreased NMDA receptor density has been described in most of the cortical areas, striatum and hippocampus (Petersen and Cotman, 1989; Miyoshi et al., 1990; Tamaru et al., 1991; Wenk et al., 1991; Cohen and Müller, 1992; Magnusson and Cotman, 1992, 1993; Cimino et al., 1993; Castorina et al., 1994; Serra et al., 1994; Nicolle et al., 1996; Wardas et al., 1997; Mitchell and Anderson, 1998). The magnitude of this decrease is consistently reported as between 20 and 50%. The decrease has been reported in several species (rats and mice) and strains of rodents (Wistar and Fischer-344 rats; BALB/c, C57B1 and NMR1 mice), independently of the binding assays used. In agreement with the decrease in the NMDA receptor density, a decrease in NMDA receptor mediated response has also been described in these cerebral structures (Baskys et al., 1990; Gonzales et al., 1991; Cepeda et al., 1996). However, along with these changes, an increase in the affinity of the NMDA receptor for glutamate (Cohen and Müller, 1992) as well as changes in the influence of modulatory sites on the NMDA receptor complex with age have also been reported (Miyoshi et al., 1990; Piggott et al., 1992).

Decreases in glutamatergic AMPA receptor density have been reported in frontal and parietal cortices of mice (Magnusson and Cotman, 1993), but not in the cerebral cortex of rats (Tamaru et al., 1991; Cimino et al., 1993). In the hippocampus, different subregions seem to change differently with age. Thus, an age-related decrease in AMPA receptor density has been reported in certain, but not all areas of the hippocampus (Cimino et al., 1993; Magnusson and Cotman, 1993), although one study described no changes in the different subregions of the hippocampus (Nicolle et al., 1996). In accordance with the decrease in AMPA receptor density described in the CA1 area of the hippocampus (Magnusson and Cotman, 1993), there is also a decrease in depolarization in response to AMPA application in this area of the brain (Barnes et al., 1992).

Of interest is a recent report showing that the decrease of NMDA and AMPA receptors in the hippocampus was significantly correlated with age-related declines in learning (Magnusson, 1998).

Regarding the glutamatergic kainate receptor, there have been reports of both decreases (Magnusson and Cotman, 1993) and no changes (Tamaru et al., 1991; Nicolle et al., 1996) in the density of kainate binding with age in the cerebral cortex and the hippocampus of the rat. Binding to glutamatergic metabotropic receptors (type 1 and 2) seems to be maintained during aging (Magnusson, 1997) and the

Table 5
Changes in glutamate receptors during aging

Brain area	Animals	Age (months)	Receptor	Changes	References
Whole cerebral cortex	Fischer-344 rats	2, 7, 29	NMDA	↓29% in density, $P<0.01$ Not significant in affinity	(Tamaru et al., 1991)
Whole cerebral cortex	Wistar–Kyoto rats	2, 6, 12, 18, 24	NMDA	↓13–23%, $P<0.05$	(Cimino et al., 1993)
Forebrain	NMR1 mice	3 vs. 20	NMDA	↓33% in density, $P<0.001$ ↑4-fold in affinity, $P<0.01$	(Cohen and Müller, 1992)
Anterior cortex	Fischer-344 rats	5 vs. 24	NMDA	$\downarrow \approx 40\%$, $P<0.01$	(Wenk et al., 1991)
Posterior cortex	Fischer-344 rats	5 vs. 24	NMDA	$\downarrow \approx 60\%$, $P<0.01$	(Wenk et al., 1991)
Frontal cortex	Fischer-344 rats	2, 5, 13, 21	NMDA	↓60%, $P<0.01$	(Miyoshi et al., 1990)
Frontal cortex	C57131/6Nia mice	3, 10, 30	NMDA	$\downarrow \approx 30\%$, $P<0.01$	(Magnusson and Cotman, 1992)
Frontal cortex	Balb/c mice	3 vs. 30	NMDA	↓	(Magnusson and Cotman, 1993)
Frontal cortex	Fischer-344 rats	4 vs. 24	NMDA	↓39% in density, $P<0.01$ Not significant in affinity	(Castorina et al., 1994)
Parietal cortex	Fischer-344 rats	2, 5, 13, 21	NMDA	↓51%, $P<0.01$	(Miyoshi et al., 1990)
Parietal cortex	C57B1/6Nia mice	3, 10, 30	NMDA	$\downarrow \approx 35\%$, $P<0.01$	(Magnusson and Cotman, 1992)
Parietal cortex	Balb/c mice	3 vs. 30	NMDA	↓	(Magnusson and Cotman, 1993)
Occipital-temporal cortex	C57B1/6Nia mice	3, 10, 30	NMDA	Not significant	(Magnusson and Cotman, 1992)
Hippocampus	Fischer-344 rats	2, 5, 13, 21	NMDA	↓47–51%	(Miyoshi et al., 1990)
Hippocampus	Fischer-344 rats	4 vs. 24	NMDA	↓26% in density, $P<0.001$ Not significant in affinity	(Castorina et al., 1994)
Hippocampus	C57B1/6Nia mice	3, 10, 30	NMDA	$\downarrow \approx 10$–20%, $P<0.01$	(Magnusson and Cotman, 1992)
Hippocampus	Fischer-344 rats	2, 7, 29	NMDA	↓24% in density, $P<0.01$ Not significant in affinity	(Tamaru et al., 1991)
Hippocampus	Fischer-344 rats	5 vs. 24	NMDA	$\downarrow \approx 50\%$, $P<0.01$	(Wenk et al., 1991)
Hippocampus	Long–Evans rats	4 vs. 24–25	NMDA	Not significant	(Nicolle et al., 1996)
Striatum	Fischer-344 rats	2, 5, 13, 21	NMDA	↓67%, $P<0.01$	(Miyoshi et al., 1990)
Striatum	Wistar–Kyoto rats	2, 6, 12, 18, 24	NMDA	↓21–35%, $P<0.01$	(Cimino et al., 1993)

Table 5 (*Continued*)

Brain area	Animals	Age (months)	Receptor	Changes	References
Striatum	Fischer-344 rats	4 vs. 24	NMDA	↓35% in density, $P<0.001$ Not significant in affinity	(Castorina et al., 1994)
Striatum	Long–Evans rats	4 vs. 24–25	NMDA	↓43%, $P<0.05$	(Nicolle et al., 1996)
Striatum (caudate)	C57B1/6Nia mice	3, 10, 30	NMDA	↓ ≈ 40%, $P<0.01$	(Magnusson and Cotman, 1992)
Striatum (caudate)	Fischer-344 rats	5 vs. 24	NMDA	↓ ≈ 50%, $P<0.01$	(Wenk et al., 1991)
Whole cerebral cortex	Fischer-344 rats	2, 7, 29	AMPA	Not significant	(Tamaru et al., 1991)
Whole cerebral cortex	Wistar–Kyoto rats	2, 6, 12, 18, 24	AMPA	Not significant	(Cimino et al., 1993)
Hippocampus (CA 1)	Wistar–Kyoto rats	2, 6, 12, 18, 24	AMPA	Not significant	(Cimino et al., 1993)
Hippocampus (CA 3)	Wistar–Kyoto rats	2, 6, 12, 18, 24	AMPA	↓16–29%, $P<0.05$	(Cimino et al., 1993)
Hippocampus (dentate gyrus)	Wistar–Kyoto rats	2, 6, 12, 18, 24	AMPA	↓16–25%, $P<0.05$	(Cimino et al., 1993)
Hippocampus	Long–Evans rats	4 vs. 24–25	AMPA	Not significant	(Nicolle et al., 1996)
Striatum	Wistar–Kyoto rats	2, 6, 12.,18, 24	AMPA	Not significant	(Cimino et al., 1993)
Hippocampus	Long–Evans rats	4 vs. 24–25	Kainate	Not significant	(Nicolle et al., 1996)
Hippocampus	Fischer-344 rats	2, 7, 29	Kainate	Not significant	(Tamaru et al., 1991)

responses mediated by these receptors remained unaltered in the hippocampus of aged animals (Jouvenceau et al., 1997).

In contrast to the reported decrease in the density of NMDA and AMPA receptors in cortex and hippocampus, several electrophysiological studies have described no changes in neuronal sensitivity to iontophoretic applications of glutamate with age in these areas of the brain (Lippa et al., 1981; Rao et al., 1993; Abdulla et al., 1995). This could be due to an increase of the affinity of glutamate for its receptors that compensates for the decrease in the number of receptors. In striatum, however, there are reports of a decrease of the neuronal responsiveness to glutamate (Cepeda and Levine, 1991; Cepeda et al., 1996).

3.5. Summary of the effects of aging on brain glutamatergic system

Several conclusions could be drawn from the data reviewed here on the effects of aging on brain glutamatergic system. First, most of the in vitro and in vivo studies on basal and stimulated release of glutamate in the cerebral cortex, hippocampus and striatum show no changes during aging, which suggests that the potentiality of glutamatergic terminals to release glutamate is unaltered. However, the stability of the release of glutamate should be interpreted with caution, since the concentration of glutamate sampled in most of the studies (slices, push–pull, microdialysis) is the result of a balance between the release and uptake processes. Thus, the reported age-related decrease in the capacity of glutamate uptake in glutamatergic terminals may compensate for changes in glutamate release. Moreover, the increase in the number and/or activity of astrocytes during aging is a confounding factor which could obscure the significance of the changes in glutamate release and uptake. Second, a consistent decrease in the number of glutamatergic receptors, mainly of the NMDA type, occurs in most of the cerebral areas of aged rodents, the magnitude of this decrease being estimated at 20–50%. This effect of aging on glutamatergic receptors would suggest that changes occur in glutamate neurotransmission in the brain.

4. Interactions of glutamate with other neurotransmitter systems

Over 30 years ago, clinical data suggested the existence of a balance or interaction between dopamine and acetylcholine as the basis of striatal functions (Barbeau, 1962), but this type of interaction was revised after the finding that glutamate also interacts with dopamine in this area of the brain (see e.g. Giorguieff et al., 1997). Since then, the concept of a complex reciprocal modulation of neural transmission among a number of neurotransmitters, including glutamate, dopamine, acetylcholine, GABA and neuropeptides, has emerged to give a better understanding of the physiology of specific circuits of the brain (Di Chiara et al. 1994; Mora et al., 1999). Although the nature of these reciprocal interactions among neurotransmitters in areas of the brain, such as prefrontral cortex and striatum, is not well understood, its dysfunction has been implied in drug addiction and in several

neurological disorders such as Parkinson's disease and schizophrenia (Carlsson and Carlsson, 1990; Grace, 1991; Greenamyre, 1993; Del Arco et al., 1998, 1999). Moreover, it has been suggested that a disruption in the fine adjustment of these neurotransmitter interactions could be at the basis of the functional decreases found during aging of the brain (Mora, 1991).

In recent years, several in vitro and in vivo studies have reported a decrease or lack of the modulation by different neurotransmitters of stimulated glutamate release in different areas of the brain during aging (Donzanti et al., 1993; Murray et al., 1997; Corsi et al., 1997). Thus, Donzanti et al. reported that activation of dopamine D2-receptor inhibits glutamate release evoked by potassium in striatal synaptosomes of young Fisher-344 rats. In contrast, this inhibition of glutamate release was not evoked in 24 to 26-month-old rats (Donzanti et al., 1993). In line with these results are those reported by Murray et al., who also showed than in old rats (22 months of age) there is a lack of inhibition by interleukin-1 of potassium-evoked release of glutamate in hippocampal synaptosomes of young rats (Murray et al., 1997).

In an in vivo study in young Wistar rats, Corsi et al. (1997) reported that potassium-evoked glutamate release in the striatum was potentiated by an adenosine antagonist, effects which were no longer produced in old rats (22 months) (Corsi et al., 1997). In contrast, these same authors showed that the effects of an adenosine agonist on basal and potassium-evoked glutamate release did not change during aging (Corsi et al., 1999). An in vivo study in our laboratory reported the loss in old (24 months) Wistar rats of the ability of melatonin to evoke glutamate release when the dopamine system is activated (Expósito et al., 1995).

Other studies have evaluated the effects of aging on the responses induced by glutamate (Cepeda and Levine, 1991; Gonzales et al., 1991; Pazzagli et al., 1995; Cepeda et al., 1996). A reduction was reported in aged Fischer-344 rats of the ability of dopamine to modulate striatal neuron responses induced by cortical stimulation in vivo (Cepeda and Levine, 1991) or activation of glutamatergic receptors by NMDA or glutamate itself in vitro (Cepeda et al., 1996). Gonzales et al. reported that dopamine release from striatal slices evoked by NMDA is lower in aged (24–28 months) than in young (3–5 months) Fischer-344 rats (Gonzales et al., 1991). In contrast, activation of metabotropic receptors in vivo increased dopamine release in prefrontal cortex of aged rats (24 months) but had no effect on young rats (3 months) (Pintor et al., 1998). An in vivo study also reported that glutamate is responsible for much of the basal striatal adenosine in old (20–22 months) but not in young (3 months) Wistar rats (Pazzagli et al., 1995).

In a recent series of in vivo experiments, we investigated the effects of aging on the glutamate–dopamine interaction in striatum and prefrontal cortex of Wistar rats (Porras and Mora, 1995; Porras et al., 1997). Interaction of neurotransmitters in striatum and prefrontal cortex has been the focus of intensive research (see e.g. Expósito et al., 1994; Sanz et al., 1997; Segovia et al., 1997; Segovia and Mora, 1998, for reviews see Di Chiara et al., 1994; Lannes and

Micheletti, 1994; Mora and Porras, 1994; Mora et al., 1999). These areas are of relevance for studies of brain aging due to their involvement in motor functions and in cognition, emotion and motivation, all of which deteriorate with age (see e.g. Ingram, 1985; Murray and Waddington, 1991; Deptula et al., 1993). In our studies, the intracerebral perfusion of a dopamine agonist, apomorphine, produced an increase of extracellular glutamate in striatum and prefrontal cortex of young rats (2–3 months), whereas a decreased response of glutamate was obtained in middle-aged (12–14 months) and aged (24–26 months) rats, which suggests a deterioration in glutamate–dopamine interaction with age (Porras and Mora, 1995; Porras et al., 1997). These data may be explained by the decrease in the number of dopamine receptors or the alterations in dopamine receptor-mediated responses (Joyce et al., 1986; Hyttel, 1987; Han et al., 1989; Morelli et al., 1990; Murray and Waddington, 1991).

We have recently used an original approach to study in vivo the endogenous interactions of glutamate with other neurotrasmitter systems in several areas of the brain (Segovia et al., 1997, 1999a,b; Del Arco and Mora, 1999; Mora et al., 1999, 2000). In young rats, intracerebral perfusion (microdialysis) of an inhibitor of glutamate uptake increases the extracellular concentrations of endogenous glutamate in a range that allowed a study of the synaptic and extrasynaptic interactions of glutamate with dopamine and GABA. In striatum and nucleus accumbens, the increase in endogenous glutamate produced a release of dopamine and GABA that was attenuated by antagonists of NMDA and AMPA/kainate receptors (Segovia et al., 1997, 1999a; Segovia, 1999). Moreover, the increases of glutamate were correlated with the increases of dopamine and GABA (Segovia et al., 1997, 1999a). When these studies were extended to aging (Segovia, 1999; Segovia et al., 1999a Mora et al., 2000), we found that the effects of endogenous glutamate on dopamine and GABA in striatum did not change during aging. On the contrary, in the nucleus accumbens, there was an age-related reduction of the increases of dopamine produced by glutamate. The effects of glutamate on GABA tended to be higher in the nucleus accumbens. These findings suggest that the changes in glutamate/dopamine and glutamate/GABA interaction during the normal process of aging show a dorso-ventral pattern in the basal ganglia, with changes in the ventral (nucleus accumbens) but not in the dorsal striatum (Segovia, 1999; Segovia et al., 1999a).

As shown above, our studies of glutamate interactions during aging suggest that although the basal release of glutamate is maintained, the effects of glutamate on other neurotransmitter systems may change as a consequence of aging (Fig. 3). These findings would be in agreement with studies reporting a decrease in the number of glutamate receptors (see above). Thus, this new perspective of an interaction among different neurotransmitters provides a better understanding of the neurochemical substrates of functional deficits during aging. The approach developed in our laboratory to study in vivo endogenous interactions among several neurotransmitters is proving to be a powerful tool to investigate the changes of these interactions in specific circuits of the brain during aging.

5. Future perspectives

Studies on the interaction between different neurotransmitter systems (i.e. glutamate and dopamine) in aging are providing new clues to understand the age-related changes in specific circuits of the brain. Specifically the interaction between glutamate, dopamine and GABA could be of interest to understand the functional neurochemical substrates of aging as it has been useful to understand the neuropathology of Parkinson's disease. In fact, this type of interaction correlated with behavioral parameters could provide significant advances in our understanding of aging. This approach might also be the basis for the development of therapeutical

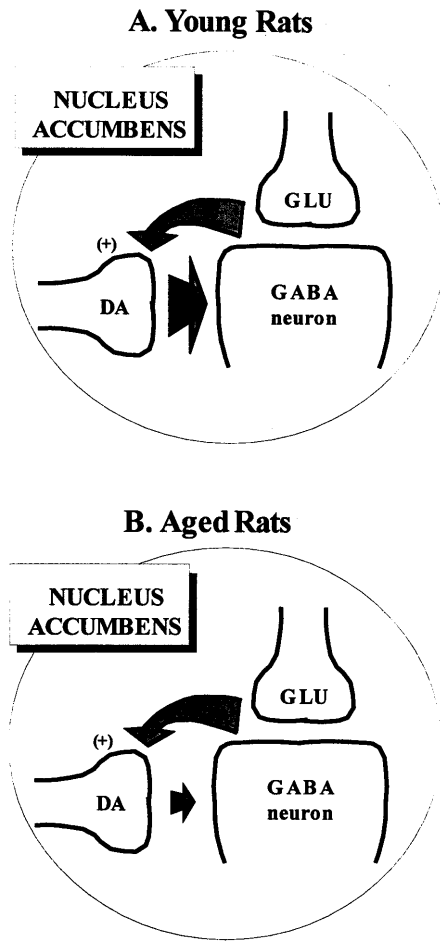

Fig. 3. Summary of the effects of aging on glutamate interactions in the nucleus accumbens: (A) Glutamate released from glutamate terminals could act extrasynaptically on dopamine terminals producing a release of dopamine that in turn could act on GABA neurons. (B) In aged rats, unaltered glutamate release produces less dopamine and GABA responses (see Segovia, 1999 for details).

tools aimed not only to compensate deficits of just one single neurotransmitter, but to rebalance the possible deficits in the interaction of multiple neurotransmitters in a specific circuit of the brain.

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