Chapter 12
Neonatal Excitotoxicity Triggers Degenerative Processes Related to Seizure Susceptibility and Pharmacoresistance

Mónica E. Ureña-Guerrero, Antoni Camins-Espuny, and Carlos Beas-Zárate

Abstract Neuronal damage and seizures are two closely related events, not only reciprocally as cause and effect but also through the cellular mechanisms and signaling pathways that they share throughout the degenerative processes that trigger them or are triggered by them. Therefore, increases in extracellular levels of the excitatory neurotransmitter glutamate, overactivation of its receptors, excessive neuronal excitation, and neuronal death by excitotoxicity have been described as part of these processes. Our group has shown that the excitotoxicity induced by monosodium glutamate (MSG) in the early stages of development produces significant modifications in the glutamatergic and GABAergic neurotransmission systems. In addition, an increased seizure susceptibility in adulthood has been observed after neonatal MSG treatment, particularly when the potassium channel blocker 4-aminopyridine or the gamma-aminobutyric acid (GABA) antagonist iodide-methyl-bicuculline is used as convulsive drugs, but not when the selective glutamate agonist N-methyl-D-aspartate (NMDA) is used. Throughout this chapter, the topics mentioned above and the hypothesis that neonatal excitotoxic damage can induce some type of drug resistance to NMDA analogs will be discussed in detail.

Keywords Excitotoxicity · Monosodium glutamate · Seizure susceptibility · NMDA receptor · Pharmacoresistance

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12.1 Introduction: The Relationship Between Excitotoxicity and Seizure Susceptibility Through Amino Acid Neurotransmitters

Although more than a hundred substances appear to act as neurotransmitters, two small molecules are particularly important in the central nervous system (CNS) of mammals: glutamate and GABA (gamma-aminobutyric acid); both are highly concentrated amino acids in the brain and are also biochemically related to each other, but in general, in adulthood, they have opposite effects on neuronal activity (Purves et al. 2001; Hassel and Dingledine 2006; Olsen and Betz 2006; Deutch and Roth 2008). Glutamate is a dicarboxylic amino acid negatively charged at physiologic pH, synthetized by the phosphate-activated glutaminase (PAG) enzyme that hydrolyzes the amine group of the glutamine in a phosphate-dependent manner, and it is considered the main excitatory neurotransmitter in the nervous system of vertebrates (Hassel and Dingledine 2006; Rowley et al. 2012). Also, recently glutamate has been proposed as a metabolic hub linking glucose and amino acid metabolism with synaptic transmission (Andersen et al. 2021). In contrast, GABA is a neutral amino acid, synthetized by the glutamic acid decarboxylase (GAD) enzyme that hydrolyzes the alpha-carboxyl group of glutamate, and it is considered the main inhibitory neurotransmitter in the nervous system of mature mammals (Olsen and Betz 2006; Rowley et al. 2012). Both glutamate and GABA are considered as classical neurotransmitters because the mechanisms involved in its synthesis, vesicular packing, release, postsynaptic receptors, synaptic inactivation, and neuronal pathways have been clearly identified in the CNS (Deutch and Roth 2008; Rowley et al. 2012). In addition, receptor specifically sensitive to each of the two neurotransmitters coexist in virtually all structures, regions, and developmental stages of the CNS (Ben-Ari 2001; Manet and Represa 2007; Deutch and Roth 2008; Aronica et al. 2011) and, interestingly, it has been shown that also GABA and glutamate can be co-released in some synapses (Gundersen 2008; Root et al. 2014). However, a particular consideration must be made, in early stages of development, when neurons have not yet established definitive synaptic contacts, GABA induces neuronal excitation and exerts trophic effects by mechanisms that include both the reverse electrochemical potential of chloride and extrasynaptic $GABA_A$ receptors (Ben-Ari 2001; Ben-Ari et al. 2007; Jensen 2011; Cellot and Cherubini 2013).

According to the essential roles of GABA and glutamate, it is evident that any significant alteration in the dynamic balance between them could lead to some pathological conditions (Martisova et al. 2012; Rowley et al. 2012; Andersen et al. 2021; Sarlo and Holton 2021; Sood et al. 2021). Thus, both experimental and clinical trials have confirmed the hypothesis that an increase in glutamate-mediated neuronal excitation or a deficiency in GABA-mediated neuronal inhibition in adulthood could increase the risk of seizures, and it is related to epilepsy (Mares and Kubová 2008; Werner and Coveñas 2011; Rowley et al. 2012; Sood et al. 2021). In general, seizures have been associated with elevated glutamate levels or reduced GABA levels in the brain (Morales-Villagran and Tapia 1996; Tapia et al. 1999;

Wilson et al. 1996; López-Pérez et al. 2010; Sarlo and Holton 2021). Also, seizures can be induced by glutamate agonists (Kohl and Dannhardt 2001; Vincent and Mulle 2009) and controlled by their antagonists (Morales-Villagran et al. 1996; Kohl and Dannhardt 2001). Otherwise, seizures can be promoted or diminished by GABA antagonists (Sperk et al. 2004; Löscher 2011) or agonists (Tolman and Faulkner 2009; Biagini et al. 2010), respectively. In addition, several alterations in the glutamatergic and GABAergic neurotransmissions also seem to be linked to the seizure activity (Treiman 2001; Mares and Kubová 2008; Werner and Coveñas 2011; Rowley et al. 2012; Sood et al. 2021). At this point, it is important to clarify that although GABA and glutamate play a fundamental role in seizure activity, other neurotransmitters and neuromodulators also have relevant implications in epilepsy (Manent and Represa 2007; Mares and Kubová 2008; Biagini et al. 2010; Werner and Coveñas 2011), one of the most complex neurological disorders. Furthermore, because GABA-mediated neuronal excitation seems to be a triggering condition for neonatal seizures (Ben-Ari et al. 2007; Jensen 2009; Briggs and Galanopoulou 2011; Cellot and Cherubini 2013; Khazipov et al. 2015), it has been hypothesized that immaturity in GABAergic signaling leading to neuronal excitation may also be a determining condition for seizure activity and epilepsies at other ages (Muñoz et al. 2007; Khazipov et al. 2015; Löscher et al. 2020; Liu et al. 2020).

On the other hand, an excessive neuronal excitation mediated by amino acids leads to neuronal death through a process known as excitotoxicity (Dodd 2002; Babot et al. 2005; Dong et al. 2009, Zhao et al. 2011). Thus, during seizure activity, increased extracellular levels of glutamate and GABA can lead to both excessive neuronal excitation and seizure-mediated excitotoxic neuronal death. In addition, the neuronal loss by whatever degenerative process in specific areas of the brain may induce seizures (Fujikawa 2005; Vincent and Mulle 2009; Chen et al. 2010; Niquet et al. 2012). Then, the relationship between seizures and excitotoxicity is closely reciprocal, and the control of one any of them could lead to control of both.

12.2 Glutamate-Mediated Excitotoxicity and Neuronal Death in Neurological Illnesses

The term “excitotoxicity” was coined by J.W. Olney to refer to neuronal death caused by overactivation of glutamate-sensitive receptors (Olney et al. 1971). This kind of death was observed for the first time, during the experimental application of monosodium glutamate (MSG) in high concentrations to treat the retinal atrophy, increasing the neuronal excitation (Lucas and Newhouse 1957). Subsequently, glutamate-mediated excitotoxicity was observed in several regions of the brain and was also related to the overexpression of glutamate receptors (Olney 1971; Garattini 1979; Choi and Rothman 1990; Young et al. 2004; Choi 2020). Now the term is applied to the neuronal death produced by a neuronal sustained excitation triggered by the overactivation of the glutamate receptors or by other mechanisms, among

which the GABA receptors overactivation may be implicated, particularly when their activation leads to neuronal excitation (Nuñez et al. 2003; Zhao et al. 2011). However, the excitotoxicity triggered by glutamate, or its analogs, is the best-known excitotoxic process, and it has been extensively associated with the neuronal death observed in several neuropathological conditions, including epilepsy (Lipton and Rosenberg 1994; Caudle and Zhang 2009; Dong et al. 2009; Choi 2020).

12.2.1 Glutamate Receptors

The excitatory glutamate effects depend on its specific interaction with cell membrane receptors, functionally classified as ionotropic (iGluR) and metabotropic (mGluR) glutamate receptors, which act as ligand-gated ion channels or as G protein-coupled receptors, respectively (Hassel and Dingledine 2006). In general, the ionotropic glutamate receptors mediate the neuronal fast depolarization allowing the Na^+ and Ca^{2+} influx and the K^+ efflux through the same ionic pore; and they are classified according to their affinity to specific exogenous agonists in sensitive receptors to N-methyl-D-aspartate (NMDAR), alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPAR), and kainic acid (KAR) (Watkins and Olverman 1987; Kohl and Dannhardt 2001; Simeone et al. 2004; Vincent and Mulle 2009; Hansen et al. 2021). Structurally, they are oligomeric macromolecular complexes formed classically by four polypeptide subunits, each of which contains an amino-terminal extracellular domain, followed by a transmembrane domain (TM1), a loop partially embedded in the membrane cytosolic face (TM2), other two transmembrane domains (TM3-4) and the carboxy-terminal intracellular domain (Simeone et al. 2004; Vandenberghe and Bredt 2004; Flores-Soto et al. 2013). The endogenous ligand glutamate interacts specifically in the neighborhood between the amino-terminal loop and the extracellular spacer loop of TM3 and TM4 (Wollmuth and Sobolevsky 2004; Flores et al. 2013) (Fig. 12.1). For each type of iGluR there are several families of subunits that, differentially distributed, may originate receptors functionally different, but activated for the same endogenous ligand, glutamate (Holopainen and Laurén 2012; Simeone et al. 2004; Wollmuth and Sobolevsky 2004). Additionally, by sequence homology, a fourth iGluR type called the GluD (or delta) receptor has been identified, but its specific endogenous ligand and whether it forms functional ion channels remains to be established (Hansen et al. 2021).

The NMDAR is characterized by its voltage dependency and high permeability to Ca^{2+}, which according to its composition in subunits, could be slowly or rapidly inactivated (Popescu and Auerbach 2003; Simeone et al. 2004; Hansen et al. 2021). It has multiple pharmacological regulatory sites, described as binding sites for (1) L-glutamate as a transmitter or endogenous ligand, and also for its competitive agonists and antagonists; (2) glycine or D-serine as coagonists; (3) phencyclidine and dizocilpine (MK801) as channel blockers; (4) Mg^{2+} as channel blocker that can be removed by depolarization; (5) Zn^{2+} as positive modulator; (6) polyamines as positive or negative modulators, depending on the compound and their concentration;

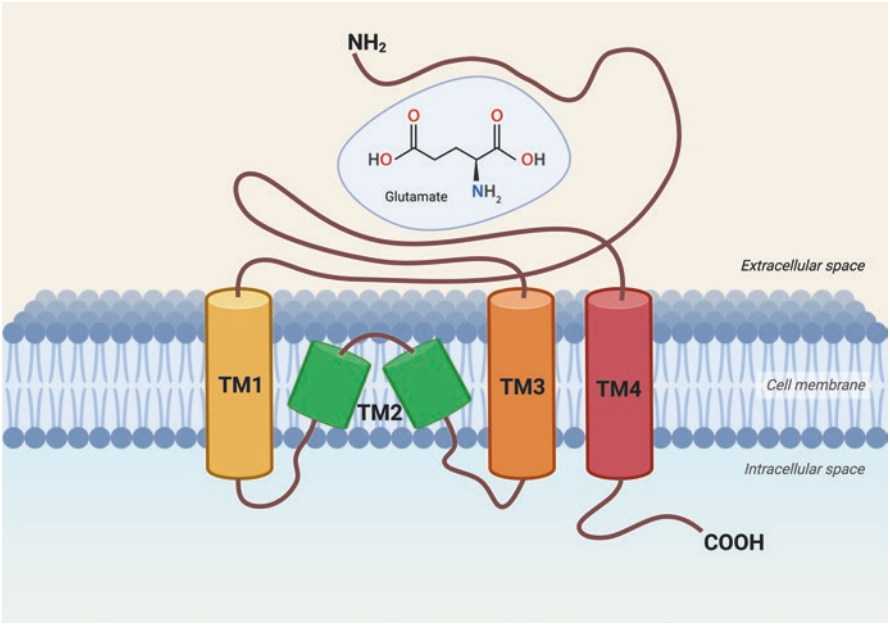

Fig. 12.1 Conformational distribution of transmembrane domains and extracellular and intracellular loops of ionotropic glutamate receptors subunits showed schematically. The extracellular loops build the binding site for glutamate, which may be exchanged by the glutamate agonist analogs in non-NMDAR and by glycine in the NR1 and D-serine in the NR3 subunits of NMDAR

and (7) a site sensitive to redox changes. Structurally, this receptor is an obligated heterotetramer conformed by combinations of the NR1 subunit (existing in eight edition variants) with the NR2A-D, NR3A-B, or both subunits, where the presence of NR1 determines the existence of a functional ion channel, while NR2A-D and NR3A-B modify the electrophysiological properties of the channel. Because each subunit family is sensitive to different agonists, NR1 to glycine, NR2 to glutamate, and NR3 to D-serine, NMDAR activation requires more than one agonist to be activated (Popescu and Auerbach 2003; Simeone et al. 2004; Wollmuth and Sobolevsky 2004; Holopainen and Laurén 2012; Flores-Soto et al. 2013; Hansen et al. 2021) (Fig. 12.2).

Non-NMDA receptors (AMPAR and KAR) are membrane voltage-independent, highly permeable to Na^+, and respond to glutamate faster than NMDARs, with which they coexist on most postsynaptic membranes (Holopainen and Laurén 2012; Hansen et al. 2021). The AMPAR also recognizes kainic acid but with low affinity compared to KAR. It is conformed as homomeric or heteromeric tetramer from the GluR1-4 subunits, whose mRNA splice variants, and in particular Q/R site editing, can change ligand selectivity as well as channel permeability and kinetic properties, leading to Ca^{2+} influx (Bettler and Mulle 1995; Simeone et al. 2004; Vandenberghe and Bredt 2004; Vincent and Mulle 2009). Besides, homomeric and heteromeric

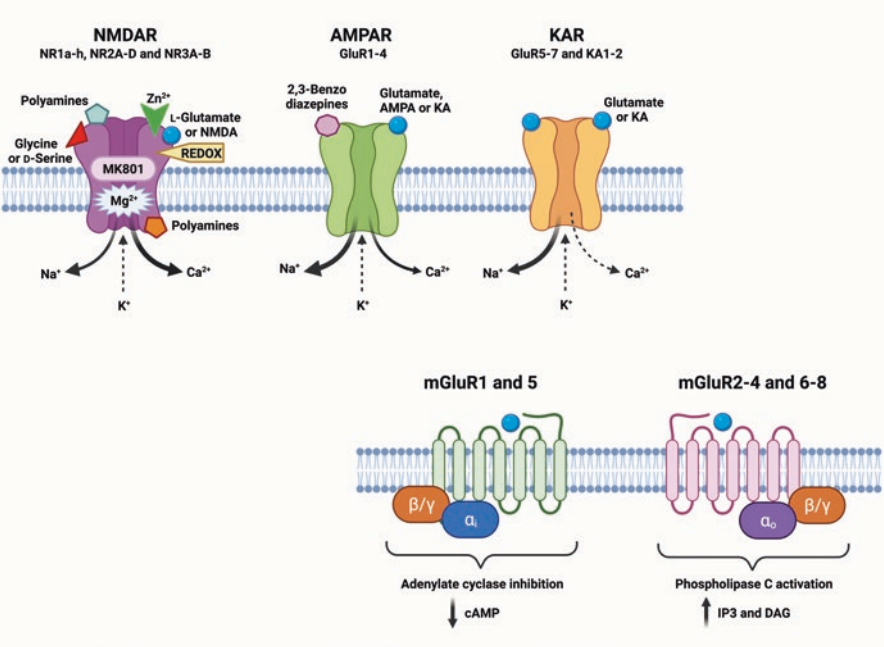

Fig. 12.2 Pharmacological binding sites, conformational subunits, and responses of the glutamate receptors showed schematically. The intensity and continuity of arrows are associated with the amplitude of the ionic currents triggered through each ionotropic glutamate receptors when they are activated for their agonists (upper panel). In the metabotropic glutamate receptors, different intracellular messengers are activated for each subtype (bottom panel)

tetramers of GluR5-7 with KA1-2 proteins build KARs, which show a high affinity by kainic acid being predominantly permeable at Na^+ (Bettler and Mulle 1995; Vincent and Mulle 2009) (Fig. 12.2).

On the other hand, mGluRs exist in homo- or heterodimeric associations, where each polypeptide contains seven helical segments that wrap back and forth through the membrane, with the extracellular amino-terminal and the intracellular carboxyl-terminal domains unusually large in comparison with other metabotropic receptors (Kunishima et al. 2000; Simeone et al. 2004; Holopainen and Laurén 2012; Stansley and Conn 2019). Eight different mGluRs identified in the nervous system have been subdivided into three groups based on their sequence homologies and enzymatic coupling. mGluR1 and mGluR5 of group I, activate a G protein-coupled to phospholipase C activation and IP3 and DAG generation, while mGluR2-3 of group II and mGluR4,6-8 of group III inhibit the production of cAMP by inhibitory G protein activation (Kunishima et al. 2000; Holopainen and Laurén 2012) (Fig. 12.2). mGluR of the groups I and II have extrasynaptic location while group III are predominantly presynaptic, and it is generally accepted that the group I increases the neuronal excitability through inhibition of several K^+ channels, while those of

Group II and III decrease the release of neurotransmitters such as GABA and glutamate (Kunishima et al. 2000; Simeone et al. 2004; Holopainen and Laurén 2012; Stansley and Conn 2019).

Finally, the synaptic effects mediated by glutamate may also be endogenously exerted by L-aspartate, another dicarboxylic nonessential amino acid, virtually ubiquitous in the human body, but highly concentrated in the brain, and generated as an intermediary metabolite or as a neurotransmitter in different metabolic pools (Hassel and Dingledine 2006; Deutch and Roth 2008; Hansen et al. 2021).

12.2.2 Mechanisms Implicated in the Neuronal Death Produced by Glutamate

Initially, glutamate receptors activation depolarizes the plasma membrane through the influx of ions promoted by their activation. But when the activation is sustained over time, the osmotic imbalance caused by the massive influx of Na^+ and Cl^- leads to cytoplasmatic Ca^{2+} overload (Young et al. 2004; Caudle and Zhang 2009; Dong et al. 2009, Szydlowska and Tymianski 2010; Choi 2020). The influx of Na^+ alters the functionality of cotransporters, pumps, and channels that depend on its electrochemical gradient (Greene and Greenamyre 1996; Dong et al. 2009; Morrison et al. 2013). The influx of Cl^- alters several plasmatic transporters and promotes Ca^{2+}-independent glutamate release that potentiates excitotoxicity (Young et al. 2004; Babot et al. 2005; Zhao et al. 2011; Choi 2020). The cytoplasmatic Ca^{2+} overload can promote: (1) the synthesis of nitric oxide, which could reach the presynaptic glutamatergic terminal to stimulate additional glutamate release through a cGMP-dependent mechanism; (2) the generation of free radicals, such as superoxide or peroxynitrites, which promote lipid peroxidation and destabilization of cell membranes; and (3) the loss of electrochemical mitochondrial potential, that alters the oxidative phosphorylation and promotes the generation of free radicals to the point of completely invalidating mitochondrial energy metabolism. In addition, Ca^{2+} can activate various intracellular signaling pathways dependent on protein kinases and phosphatases that could promote the proteolysis of cell content (Greene and Greenamyre 1996; Montal 1998; Arundine and Tymianski 2003; Young et al. 2004; Dong et al. 2009; Szydlowska and Tymianski 2010; Choi 2020) (Fig. 12.3).

The glutamate-mediated excitotoxicity as a continuous process may be strongly acute in its initial phase and trigger neuronal death by necrosis, but it can also evolve more slowly and cause apoptosis (Young et al. 2004). In this sense, in vitro studies have shown that glutamate can produce both types of death depending on its application scheme (Bonfoco et al. 1995; Portera-Cailliau et al. 1997a, b). Thus, a brief exposition to high concentrations of glutamate could cause acute neuronal death due to early degenerative changes related to tissue inflammatory process that is characterized by being dependent Na^+ and Cl^-. Otherwise, prolonged exposure to lower concentrations of glutamate could lead to delayed neuronal death, which is

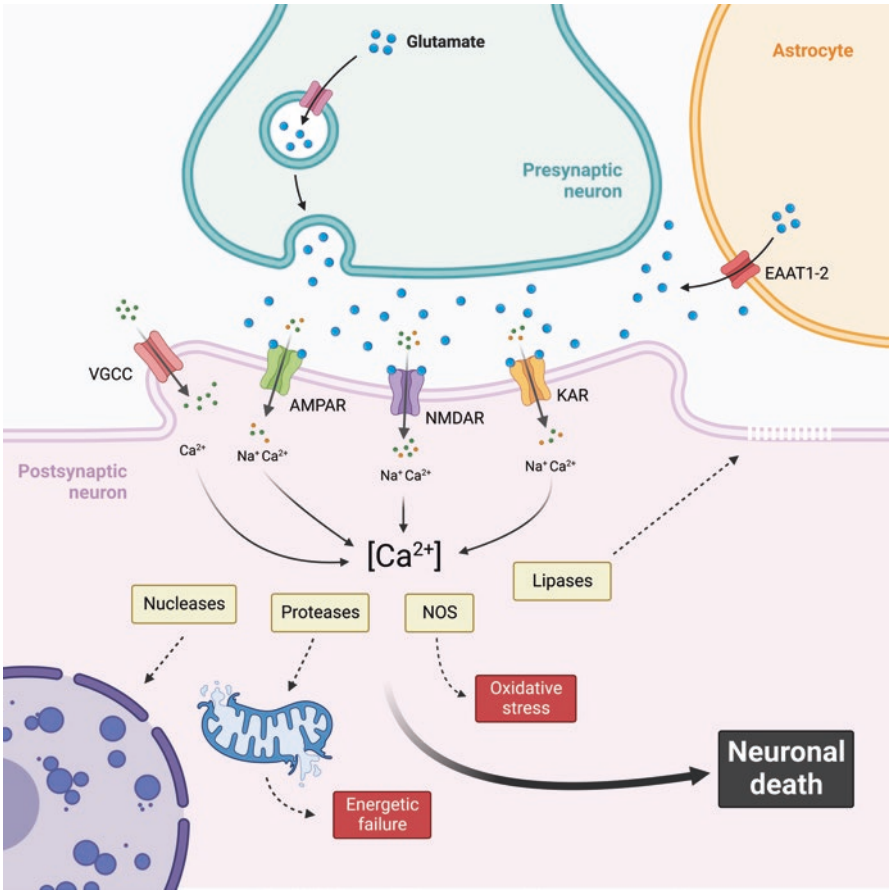

Fig. 12.3 Schematic representation of the most relevant events that lead to glutamate-induced neuronal death, involving ionic imbalance, energy failure, oxidative stress, and intracellular signaling pathways triggered by calcium overload, among other mechanisms. Death can occur by necrosis or apoptosis depending on the triggered stimulus or the initial energetic functional state. Abbreviations: VGCC voltage-gated calcium channels, NOS nitric oxide synthase, EAAT1-2 excitatory amino acid transporters 1 and 2 types

dependent on Ca^{2+} influx and requires several hours or even days to occur (Bonfoco et al. 1995; Portera-Cailliau et al. 1997a, b; Young et al. 2004). Also, it has been suggested that the glutamate-mediated degenerative process depends largely on the functional mitochondrial state and that when the cellular metabolic rate is reduced, the mitochondria is unable to maintain homeostasis of $Ca^{2+,}$ and therefore neuronal death is mainly due to apoptosis (Bonfoco et al. 1995; Portera-Cailliau et al. 1997a, b; Young et al. 2004; Niizuma et al. 2010).

12.2.3 Glutamate-Mediated Excitotoxicity and Neurological Illnesses

Studies carried out in different neural systems, both in vivo and in vitro, on glutamate-mediated excitotoxic degeneration have demonstrated that in pathological conditions, such as cerebral hypoxia-ischemia (Choi and Rothman 1990; Szydlowska and Tymianski 2010; Choi 2020), traumatic brain injury (Bramlett and Dietrich 2004; Wagner et al. 2005), epilepsy (Meldrum 1993a; Wilson et al. 1996; Friedman et al. 2003; Sarlo and Holton 2021) and domoic acid (Meldrum 1993b; Jeffery et al. 2004), glutamate concentration increases significantly in the brain, and these increases are closely related to the observed neuronal damage. Additionally, it has been proposed that excitotoxicity participates in the establishment of several neurodegenerative diseases such as Huntington's (Beal et al. 1991; Gardian and Vecsei 2004), Alzheimer's (Ferrarese et al. 2000; Hynd et al. 2004) and Parkinson's diseases (Lipton and Rosenberg 1994; Rego and Oliveira 2003; Caudle and Zhang 2009), as well as in schizophrenia (Lipton and Rosenberg 1994), among other degenerative processes. In this regard, experimental trials have shown that glutamate antagonists could protect against neuronal excitotoxic damage and control seizures, reducing neurodegenerative processes (Meldrum 1985; Morales-Villagran et al. 1996; Harty and Rogawski 2000; Löscher et al. 2020). In clinical trials, this knowledge has been applied with some success; for example, memantine, one of the therapeutic agents used recently for Alzheimer's disease, although it does not cure the disease, can slow down its progression, acting as NMDAR antagonist (Moreira et al. 2006; Supnet and Bezprozvanny 2010). Also, memantine resembles to exert positive effects on vascular dementia and Parkinson's disease (Olivares et al. 2012). Another example is dizocilpine, one NMDAR ion channel blocker that, applied in combination with nimodipine, appears to decrease the penumbra area in acute excitotoxic neuronal damage caused by a hypoxic–ischemic event, but its neuroprotective effect is variable and sometimes insignificant (Niizuma et al. 2010; Sydlowska and Tymianski 2010). In addition, perampanel, topiramate, and felbamate, which act as iGluR antagonists, appear to control some types of epilepsy, particularly focal epilepsies (Celli and Fornai 2021).

12.3 Systemic Administration of Monosodium Glutamate as Excitotoxicity Model

Although various glutamate agonists have been used to trigger excitotoxic neuronal damage, systemic administration of monosodium glutamate (MSG) is probably the best option to study the glutamate-induced neurodegenerative process in an integral manner. Through this model, it has been possible to temporally characterize the neurophysiological alterations and compensatory responses that follow the

excitotoxic insult, and to establish that most mammalian species are susceptible to the toxic effects of glutamate, and that the severity of the induced damage depends on the species, age, and gender (Garattini 1979). Thus, it is now known that the greatest susceptibility to glutamate-mediated excitotoxicity is observed in: (1) newborn male mammals compared to adults, females and other vertebrates (Garattini 1979); (2) in brain regions where the density of glutamate receptors is higher, such as the hippocampus and cerebral cortex (Meldrum 1993b; Beas-Zarate et al. 2002a; Kim et al. 2009), among others; and (3) in neural and nonneural cells that express glutamate receptors, such as GABA neurons (Reeves et al. 1987; Muller et al. 2001; Ureña-Guerrero et al. 2009), microglial cells (Brown and Neher 2010) and Bergmann glial cells (Mendez-Flores et al. 2016), among others.

The immaturity of the blood-brain barrier (Xu and Ling 1994; Ek et al. 2006; Bell et al. 2020), low glutamate uptake (Thomas et al. 2011; Rose et al. 2018), long amplitude and duration of NMDA- and voltage-gated Ca^{2+} currents (Ben-Ari 2001; Jensen 2009; Dehorter et al. 2012), and GABA-mediated excitability (Nuñez et al. 2003; Ben-Ari et al. 2007; Zhao et al. 2011), are some of the conditions associated with the high susceptibility to glutamate-mediated excitotoxicity characteristically observed in newborns. However, systemically administered MSG can also induce damage in adulthood, particularly in areas of the brain where the blood-brain barrier is deficient, such as the arcuate nucleus and other hypothalamic nuclei (Garattini 1979; Hu et al. 1998). Additionally, also it is known that glutamate-mediated excitotoxicity could be associated with seizures (Arauz-Contreras and Feria-Velasco 1984; Lopez-Perez et al. 2010), obesity (Garattini 1979; Hu et al. 1998; Kirk et al. 2009; Hernández Bautista et al. 2014; Andres-Hernando et al. 2021), migraine (Benbow et al. 2022), and learning (Ishikawa et al. 1997; Velazquez-Zamora et al. 2011) and motor impairments (Möykkynen and Korpi 2012; Firgany and Sarhan 2020) with the males being more susceptible than females, probably due to the neuroprotective effect exerted by steroids (Luoma et al. 2011).

12.3.1 Changes Induced by Systemically Administered MSG in Neonatal Rats

As mentioned above, systemic administration of MSG to newborn rodents induces acute neuronal damage and compensatory changes, which can be characterized over time. Thus, among the immediate changes, Hu et al. (1998) showed that a single MSG dose of 0.2 mg/g of body weight administered subcutaneously on postnatal day (PD) 7 in male mice, produced a 17-fold elevation of plasma glutamate levels above the initial value, which was associated with increases in the expression level of NR1 and GluR2/4 subunits, and minor but significant injury in subependymal neurons near the base of the third ventricle. More recently, it was shown via an

enzymatic biosensor implanted in the right lateral ventricle of the brain that MSG 4 mg/g of body weight administered subcutaneously to newborn male rats on PD1 increased the extracellular glutamate levels to values close to 300% above the baseline. Increases in the extracellular glutamate levels were more pronounced when the same dose of MSG was re-administrated at PD3 and PD5, but no increases in PD7 were observed after the fourth administration of the same dose of MSG. These increases in the extracellular brain glutamate levels were associated with electrographic and behavioral epileptiform activities, as well as with rises in total glutamate, glutamine, and GABA contents measured in the hippocampus 24 hours after each MSG administration (Lopez-Perez et al. 2010). In addition, using the administration scheme described above, where 4 mg of MSG per gram of body weight is subcutaneously administered to neonatal male rats four times on PD1, 3, 5, and 7 (a model implemented by our working group), the neuronal death by apoptosis was observed in CA1 and CA3 hippocampal regions, as well as in the cerebral cortex, 24 hours after the last administration (Chaparro-Huerta et al. 2002, 2005; Rivera-Cervantes et al. 2004, 2009). This neuronal loss was also associated with changes in the expression levels of NMDAR and AMPAR subunits (Rivera-Cervantes et al. 2004, 2009) and with increases in the levels of p38 kinase protein and in TNF-α proinflammatory cytokine (Chaparro-Huerta et al. 2002, 2005; Rivera-Cervantes et al. 2004, 2009).

Additionally, after neonatal MSG treatment, the loss of pyramidal (Gonzalez-Burgos et al. 2001; Beas-Zarate et al. 2002a; Velazquez-Zamora et al. 2011), GABA-positive (Ureña-Guerrero et al. 2009) and dopaminergic (Lopez-Perez et al. 2005) neurons has been observed in various brain regions of adult rats. This neuronal loss has been associated with changes in the expression level of non-NMDA and NMDA subunits (Beas-Zarate et al. 2001, 2002b, 2007) and of glutamate transporters (Medina-Ceja et al. 2012; Castañeda-Cabral et al. 2020); in the binding sites to acetylcholine, and choline acetyltransferase activity (Ortuño-Sahagun et al. 1997); as well as in dopamine receptors and transporters (Lopez-Perez et al. 2005); in the [^{3}H]-GABA release (Beas-Zarate et al. 1998) and uptake (Ureña-Guerrero et al. 2009); in glutamic acid decarboxylase activity (Ureña-Guerrero et al. 2003); and in others GABAergic markers (Ureña-Guerrero et al. 2009); all of them observed in different brain regions and ages after treatment until adulthood. Furthermore, the MSG neonatal treatment induces hyperplasia and hypertrophy on astrocytes and microglial cells in the cerebral cortex and hippocampus of adult rats (Martinez-Contreras et al. 2002; Castañeda-Cabral et al. 2020). In this point, it is important to mention that neonatal MSG treatment produces significant changes in seizure susceptibility (Ureña-Guerrero and Beas-Zarate 2006), as well as in learning capacity (Gonzalez-Burgos et al. 2001; Velazquez-Zamora et al. 2011), both of which are closely related with the modifications described above.

12.4 Changes in Adulthood Seizure Susceptibility After MSG Neonatal Treatment and Its Possible Relationship with the Pharmacoresistance

When we observed that adult rats neonatally treated with MSG developed an unusual wild running behavior after simple manipulations as cage switching, and consistent with the significant changes induced in GABAergic and glutamatergic neurotransmission systems mentioned above, we decided to characterize seizure susceptibility through some experimental models to induce convulsions. First, we used 4-aminopyridine as a generic convulsive drug that acts as a blocker of voltage-sensitive potassium channels; followed by iodide-methyl-bicuculline as GABA antagonist and NMDA as glutamate agonist, all of them administered intracerebrally into the right lateral ventricle in awake adult rats (Ureña-Guerrero and Beas-Zarate 2006). Except to NMDA, all convulsive drugs induced more severe convulsive symptoms in the MSG-treated group than in the control group. Moreover, the seizure latency was shorter, and the seizure duration was longer in the MSG-treated group than in the control group (Ureña-Guerrero and Beas-Zarate 2006; Hernandez-Ojeda et al. 2017) (Table 12.1). Intracebroventricular (i.c.v.) administration of NMDA (10 nmol) in the MSG-treated group produced repenting, intense jumps and tremors, and facial clonus and forelimb clonus. Still, the motor behavioral alterations disappeared during the first 15 minutes. They did not generate any epileptiform

Table 12.1 Minimal dose of some convulsive drugs necessary to induce severe convulsive signs in adulthood after MSG neonatal treatment

	Convulsive drugs (doses)					
	4-Aminopyridine (1, 2, 3, 4, and 5 nmol)		Iodide-methyl bicuculline (0.25, 0.5, 1, 1.5, and 2 nmol)		N-Methyl-D-Aspartate (2.5, 5, 7.5, and 10 nmol)	
Severe convulsive signs	Control	MSG-treated	Control	MSG-treated	Control	MSG-treated
Wild running	3.0	1.0	1.5	0.5	–	–
Rearing	2.0	1.0	1.0	0.25	5.0	–
Generalized tonic–clonic convulsions	4.0	2.0	2.0	1.0	5.0	–
Status epilepticus establishment	4.0	2.0	–	1.0	7.5	–
Animal death (%)[a]	4.0 (25%)	2.0 (80%)	–	1.5 (37.5%)	10 (50%)	–

Data indicate the dose at which each severe convulsive sign was observed in each experimental group. Doses tested are indicated in parentheses under each convulsive drug

[a]The percentage of animal death was estimated from eight animals for each group, convulsive drug, and dose

discharge in the hippocampus of adult rats, while in the control group, behavioral and electrographically, the NMDA injection induced generalized tonic–clonic convulsions, status epilepticus, and death (Ureña-Guerrero and Beas Zarate 2006) (Table 12.1). Interestingly, electrographic recordings of basal activity in the hippocampus and entorhinal cortex of MSG-treated adult rats were characterized by a lower net amplitude and higher average firing frequency than that observed in the control group. In addition, 3 nmol of 4-aminopyridine via i.c.v. induced a greater number of firing bursts with higher net amplitude and longer duration in MSG-treated adult rats (Hernández-Ojeda et al. 2017).

Thus, although more studies are necessary, the evidence suggests that after neonatal MSG treatment, some adaptive changes occur at the level of NMDA receptors that could generate some type of resistance to NMDA agonists. In this sense, it is important to mention that when neonatal MSG treatment is administered to male rats, the NMDAR is more abundant than the non-NMDAR (Simeone et al. 2004; Holopainen and Laurén 2012), particularly in the cerebral cortex and the hippocampus, where any electrographic epileptiform discharges were recorded after intracerebral NMDA administration in adult rats treated with MSG. In addition, experimental evidence has been demonstrated that NMDAR activation could lead to its structural and functional modification resembling any kind of "habituation ligand-receptor" or "preconditioning", where the NMDAR does not become responsive to NMDA (Boeck et al. 2004; Severino et al. 2011). Then, neonatal MSG treatment could induce a pronounced preconditioning, which seems to remain until adulthood, where NMDA i.c.v. administration does not induce the epileptiform activity observed in control rats (Ureña-Guerrero and Beas-Zarate 2006). In this sense, the NMDAR functional modifications have also been suggested in the studies where learning impairment has been reported after neonatal MSG treatment (Gonzalez-Burgos et al. 2001; Velazquez-Zamora et al. 2011). According to the last, although the pharmacoresistance in epilepsy has been primarily related to changes in the expression levels of voltage-gated sodium and calcium channels, $GABA_A$ receptor subunits, and efflux transporters (Remy and Beck 2006; Löscher et al. 2020), it is possible that MSG neonatal treatment may induce some form of pharmacoresistance, especially for anticonvulsive drugs that act on NMDAR (Celli and Fornai 2021), such as felbamate (Harty and Rogawski 2000) and lamotrigine (Wang et al. 1996). In this sense, it has been reported that a short preconditioning with NMDA is able to diminish the anticonvulsive efficacy of lamotrigine, without a significant effect on felbamate (Tomczyk et al. 2007).

Finally, the changes induced in non-NMDA receptors after MSG treatment (Rivera-Cervantes et al. 2004, 2009; Beas-Zarate et al. 2007) could be involved in seizure susceptibility, but also, they could be originating some form of pharmaresistance for the drugs acting through those receptors (Lasoń et al. 2011; Celli and Fornai 2021). Then, the changes induced by neonatal MSG treatment on glutamate receptors remain to be deeply characterized, particularly their association with a possible pharmacoresistance to NMDA.

12.5 Concluding Remarks and Perspectives

Because the increases in extracellular brain glutamate levels induced by neonatal MSG treatment resemble those seen in various neonatal neurological disorders, including hypoxic-ischemic and anoxic episodes, traumatic brain injury, and seizures, an in-depth characterization of treatment-induced changes in the brain is important to elucidate the mechanisms associated with both seizure susceptibility and drug resistance observed in humans after excitotoxic damage. We considered the pharmacological and electrophysiological characterization of glutamate and GABA receptors to be particularly important after neonatal MSG treatment, as they have been deeply implicated in seizure susceptibility and excitotoxicity, as well as in pharmacoresistance in epilepsy.

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