INSTITUTO POTOSINO DE INVESTIGACIÓN CIENTÍFICA Y TECNOLÓGICA, A.C.

POSGRADO EN CIENCIAS EN BIOLOGIA MOLECULAR

“Changes in the expression levels of glutamate transporters in a pharmacological model of schizophrenia”

Tesis que presenta

Ares Orlando Cuellar Santoyo

Para obtener el grado de

Maestro en Ciencias en Biología Molecular

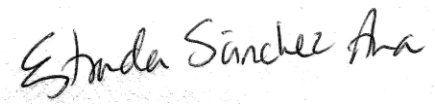
Directora de la Tesis:

Dra. Ana María Estrada Sánchez

San Luis Potosí, S.L.P., Julio 2021

Constancia de aprobación de la tesis

La tesis **"Changes in the expression levels of glutamate transporters in a pharmacological model of schizophrenia"** presentada para obtener el Grado de Maestro en Ciencias en Biología Molecular fue elaborada por **Ares Orlando Cuellar Santoyo** y aprobada el **16 de junio de 2021** por los suscritos, designados por el Colegio de Profesores de la División de Biología Molecular del Instituto Potosino de Investigación Científica y Tecnológica, A.C.



Dra. Ana María Estrada Sánchez
Directora de la Tesis

Dra. María Jazmín Abraham Juárez
Miembro del Comité Tutoral

Dr. Alberto Camacho Morales
Miembro del Comité Tutoral

Créditos Institucionales

Esta tesis fue elaborada en el Laboratorio de Neurobiología de la División de Biología Molecular del Instituto Potosino de Investigación Científica y Tecnológica, A.C., bajo la dirección de la Dra. Ana María Estrada Sánchez.

Durante la realización del trabajo el autor recibió una beca académica del Consejo Nacional de Ciencia y Tecnología (No. 753050) y del Instituto Potosino de Investigación Científica y Tecnológica, A. C.

Este trabajo fue apoyado por el proyecto CONACYT-FOSEC SEP-Investigación Básica A1-S-26479.

IPICYT

Instituto Potosino de Investigación Científica y Tecnológica, A.C.

Acta de Examen de Grado

El Secretario Académico del Instituto Potosino de Investigación Científica y Tecnológica, A.C., certifica que en el Acta 221 del Libro Segundo de Actas de Exámenes de Grado del Programa de Maestría en Ciencias en Biología Molecular está asentado lo siguiente:

En la ciudad de San Luis Potosí a los 12 días del mes de julio del año 2021, se reunió a las 16:00 horas en las instalaciones del Instituto Potosino de Investigación Científica y Tecnológica, A.C., el Jurado integrado por:

Dra. María Jazmín Abraham Juárez	**Presidenta**	**IPICYT**
Dra. Ana María Estrada Sánchez	**Secretaria**	**IPICYT**

a fin de efectuar el examen, que para obtener el Grado de:

MAESTRO EN CIENCIAS EN BIOLOGÍA MOLECULAR

sustentó el C.

Ares Orlando Cuellar Santoyo

sobre la Tesis intitulada:

Changes in the expression levels of glutamate transporters in a pharmacological model of schizophrenia

que se desarrolló bajo la dirección de

Dra. Ana María Estrada Sánchez

El Jurado, después de deliberar, determinó

APROBARLO

Dándose por terminado el acto a las 18:49 horas, procediendo a la firma del Acta los integrantes del Jurado. Dando fe el Secretario Académico del Instituto.

A petición del interesado y para los fines que al mismo convengan, se extiende el presente documento en la ciudad de San Luis Potosí, S.L.P., México, a los 12 días del mes de julio de 2021.

Dr. Marcial Bonilla Marín
Secretario Académico

Mtra. Ivonne Lizette Cuevas Vélez
Jefa del Departamento del Posgrado

IPICYT
SECRETARÍA ACADÉMICA
INSTITUTO POTOSINO DE
INVESTIGACIÓN CIENTÍFICA
Y TECNOLÓGICA, A.C.

Dedicatorias

A mis padres y hermanos, por contar con su apoyo en todo momento durante mi formación profesional.

A mis amigos, por hacer de esta etapa nada aburrida.

Agradecimientos

Agradezco al Consejo Nacional de Ciencia y Tecnología (CONACYT) por la beca otorgada Número: 753050.

Al Instituto Potosino de Investigación Científica y Tecnológica A.C.(IPICYT) por darme la oportunidad de cursar el programa de maestría en Biología Molecular.

A la Dra. Ana María, por darme la oportunidad de formar parte de su laboratorio, por la paciencia, el consejo y su grata y sincera amistad.

Agradezco a la Dra. María Jazmín Abraham Juárez, por sus consejos y por darme la oportunidad de trabajar en su laboratorio.

Agradezco al Dr. Alberto Camacho Morales, por sus sugerencias y comentarios para el desarrollo óptimo de este trabajo.

Agradezco al Dr. Victor Manuel Ruiz Rodríguez, por su apoyo técnico en la elaboración de este trabajo.

Agradezco a la Dra. Olga Araceli Patrón Soberano, por su asesoría en el manejo de equipo de microscopia y por apoyarnos en la administración del laboratorio.

Índice

Lista de tablas

Lista de figuras

Anexos

Resumen

“Cambios en los niveles de expresión de los transportadores de glutamato en un modelo farmacológico de esquizofrenia”

En la visión tradicional de la comunicación sináptica, la comunicación neuronal mediada por el glutamato o transmisión glutamatérgica se ha centrado en la actividad de las neuronas presinápticas y postsinápticas, mientras que los astrocitos aseguran un flujo exitoso de la información neuronal. Sin embrago, nuevas evidencias indican que los astrocitos poseen un papel activo en la modulación de la comunicación neuronal. En este trabajo, inicialmente se describen los componentes de la sinapsis tripartita glutamatérgica: la terminal presináptica, la postsinápsis y los astrocitos; así como la contribución de los astrocitos a la neurotransmisión glutamatérgica. Posteriormente, se exploran los efectos de la inhibición crónica de los receptores NMDA por su antagonista ketamina (30 mg / kg durante 14 días) sobre la expresión del transportador de glutamato/aspartato (GLAST) y el transportador de soluto xCT (SLC7A / xCT) en el hipocampo de ratón. La administración sistémica de ketamina durante 14 días se utiliza como modelo farmacológico para el estudio de la esquizofrenia. Los resultados indican que la administración crónica de ketamina no afecta los niveles de GLAST, pero aumenta significativamente los niveles de expresión de SLC7A / xCT. Este último transportador es un componente de la síntesis del antioxidante glutatión, lo que sugiere que el tratamiento con ketamina puede estar aumentando la formación de radicales libres. La evaluación del efecto de la ketamina sobre los transportadores de glutamato GLT-1 y EAAC1 es necesaria para para comprender el impacto de este fármaco sobre el sistema de recaptación de glutamato.

PALABRAS CLAVE: Gliotransmisión, Astrocito, Ketamina, Calcio, Glutatión e Hipocampo.

Abstract

“Changes in the expression levels of glutamate transporters in a pharmacological model of schizophrenia”

Neuronal communication mediated by glutamate or glutamatergic neurotransmission has typically centered on the activity of presynaptic and postsynaptic neurons. In the traditional view of synaptic communication, the astrocyte’s role was mostly to ensure a successful neuronal information flow. In this thesis, the components of the glutamatergic neurotransmission are described, what it is known so far about the three components: presynaptic, postsynaptic, and astrocytes -altogether known as a tripartite synapse-. Also, the contribution of astrocytes to glutamatergic neurotransmission is discussed. The chapter one explores the effects of the chronic inhibition of NMDA receptors by its antagonist ketamine (30 mg / Kg for 14 days) on the expression of glutamate/aspartate transporter (GLAST) and the solute carrier transporter xCT (SLC7A/xCT) in the mouse hippocampus. The systemic administration of ketamine during 14 days is used as a pharmacological model to study schizophrenia. The xCT transporter is a component of the synthesis of the antioxidant glutathione, suggesting that ketamine treatment may be increasing the formation of free radicals. Evaluation of the effect of ketamine on the glutamate transporters GLT-1 and EAAC1 is necessary to understand the impact of this drug on the glutamate receptor system.

KEYWORDS: Gliotransmission, Astrocytes, Ketamine, Calcium, Glutathione and Hippocampus.

Introduction.

The brain is one of the most complex organs that constitute the human body (1), and it is conformed of a great variety of cells, where neurons and glial cells stand out. The glial cells are divided into microglia (immune cells), oligodendrocytes (responsible for the myelin formation), and astrocytes. From all glial cells, the most abundant are the astrocytes (2), which participate in physiological processes, including synaptogenesis (3), modulation of synaptic transmission, neuronal plasticity (4,5), and regulation of blood flow and trafficking of small molecules and ions through the end-feet processes at the blood-brain barrier (6,7).

All the wide variety of brain functions, interactions with the environment (special senses), movements, and cognitive processes, are generated by chemical or electrical synaptic communication (8). Chemical transmission is the most abundant in the mammalian nervous system (9), and it refers to the communication between a presynaptic terminal and a postsynaptic neuron by releasing a neurotransmitter into the synaptic cleft that will bind to its receptors on the postsynaptic terminal triggering a cascade of events that will generate an electrical or chemical response (10).

Of all the neurotransmitters, glutamate is one of the most abundant, and it participates in many physiological processes such as learning, memory, neuronal development and maturation (11–13). However, glutamate might become neurotoxic if its extracellular concentration is not properly regulated. An active uptake system must control extracellular glutamate concentrations to avoid its accumulation and possible cell damage (14). Glutamate transporters located in neurons and astrocytes are responsible for the uptake of the neurotransmitter after its release (15). If the

glutamate uptake system fails, glutamate accumulation can damage cortical neurons, neurons from the retina, striatal and hippocampal neurons (16–18). Glutamate-mediated damage is known as excitotoxicity and it refers to the over-activation of glutamatergic receptors, which has been related to the pathogenesis of neurodegenerative diseases such as Alzheimer's, Huntington's, and Parkinson's diseases (19–21). Also, some mental disorders, such as schizophrenia, are related to impaired functioning of glutamatergic neurotransmission (22,23). However, although glutamate toxicity might contribute to these neuropathological conditions, the changes that lead to impaired glutamatergic neurotransmission are not fully understood.

The characteristics of glutamatergic transmission and its possible modulating role through neurons and astrocytes are explained in the next section.

Overview of the tripartite glutamatergic transmission.

Glutamate is the most important excitatory neurotransmitter in the mammalian brain, considering that more than half of the neurons release this neurotransmitter (24–26). Glutamate is synthesized in the neurons by specialized enzymes, but its metabolism also involves glial cells, specifically astrocytes (27). Astrocytes are an active component of neurotransmission and some authors include its role in the term "tripartite glutamatergic synapsis". In response to neuronal activity, astrocytes generate responses such as the elevation of intracellular calcium concentrations and the consequent gliotransmitter release, modulation of extracellular potassium

concentration, and removal of neurotransmitters. It is through these processes that astrocytes might modulate the dynamics of synaptic communication (4).

After its release, glutamate activates a wide number of ionotropic (iGluRs) or metabotropic receptors (mGluRs) (28,29). Extracellular glutamate concentration is highly regulated by Na^+-dependent an active transport system. There are five glutamate transporters: excitatory amino acid carrier 1 (EAAC1), the glutamate-aspartate transporter (GLAST/EAAT1), the glutamate transporter 1 (GLT-1/EAAT2), the excitatory amino acid transporter 4 (EAAT4), and the excitatory amino acid transporter 5 (EAAT5) (30–33).

Glutamate synthesis, packaging, and release.

Glutamate synthesis.

Since glutamate doesn't cross the blood-brain barrier, it must be synthesized in neurons from local precursors (34), and different pathways contribute to its synthesis (fig. 1). One of them is through the enzyme glutamate dehydrogenase. This enzyme catalyzes the interconversion of glutamate to α-ketoglutarate and ammonia, using NAD^+ and $NADP^+$ as a co-factor, this reaction is important for glutamate and ammonia regulation (35). There is evidence that glutamate dehydrogenase deficiency affects the glutamate and ammonia metabolisms, causing pathological changes in the brain like slowly progressive spinocerebellar ataxia, lower cranial nerve dysfunction, and various extrapyramidal manifestations (36).

Aspartate aminotransferase is another enzyme that synthesizes glutamate by a reversible process of transferring the α-amino group from aspartate or alanine to

2-oxoglutarate to form glutamate and oxaloacetate or pyruvate; this enzyme uses pyridoxal 5'-phosphate as a co-factor. A decrease of aspartate aminotransferase activity in the olfactory bulbs, cerebral cortex, and cerebellum of the rat was reported after being subjected to unilateral compression of femoral tissues (crush syndrome) (37).

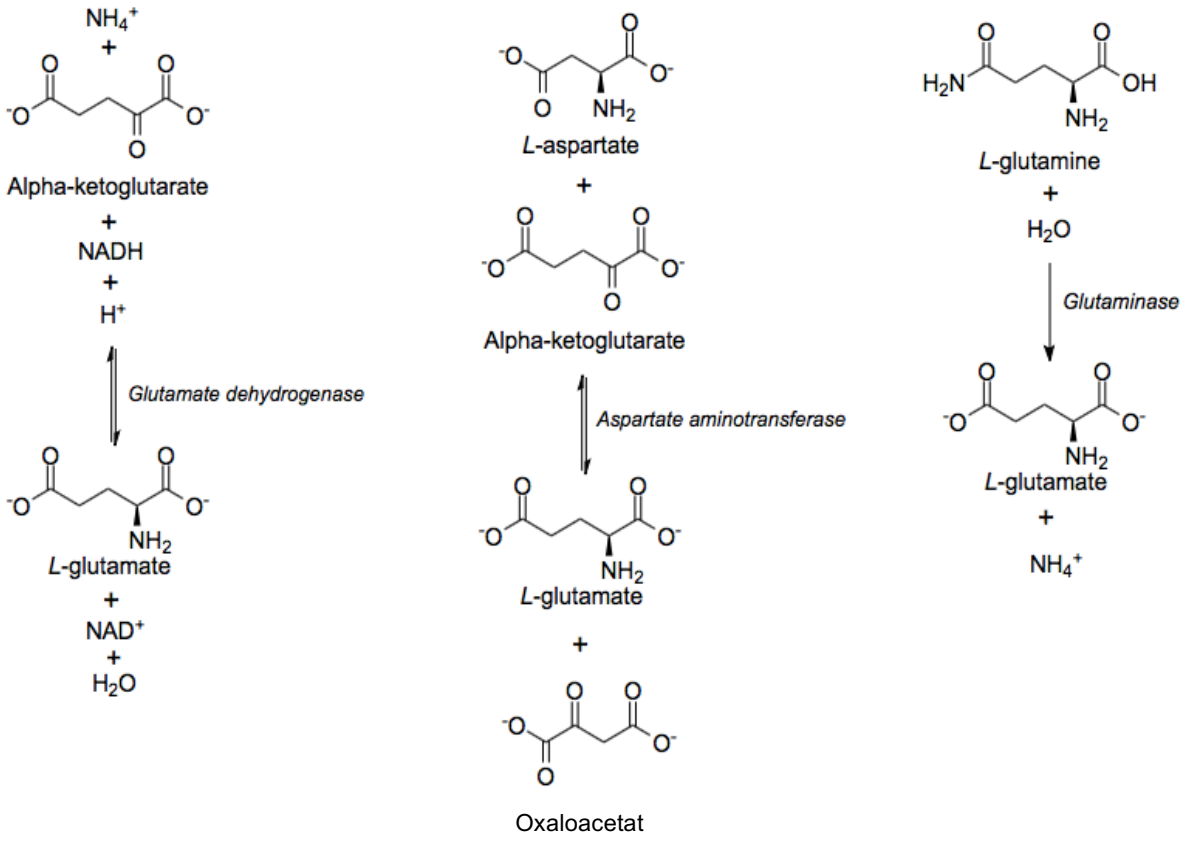


Fig. 1.- Glutamate synthesis. - Schematic representation of three main reactions that synthesize glutamate in the brain: A) Glutamate dehydrogenase, B) Aspartate aminotransferase, and C) Glutaminase (35,37,38).

One of the most important pathways to synthesize glutamate in the brain is to convert glutamine to glutamate by the enzyme glutaminase, an amidohydrolase. Two isozymes of glutaminase have been described, the liver-type (GLS2) and the kidney-type (GLS1). High levels of GLS1 are expressed in neurons (38), which can obtain glutamine from nutrients absorbed from the food and the glial cells; glutamine is transported out of the glial cell and recaptured by the neurons SLC (solute carrier) transporters. By comparing the copy number of the mRNAs encoding the SLC transporters, SLC38a is specified as the major glutamine transporter in neurons, followed by SNAT1 (39). Once inside the neuron, glutamine will be a precursor for glutamate. The relevance of glutaminase was evaluated by developing glutaminase knockout mice that showed altered respiratory phenotype, suggesting that this enzyme is important for various regulatory processes (40).

Using histochemical studies, the presence of glutamate dehydrogenase was determined in astrocytes, which has a different activity pattern according to the brain region, species, and astrocyte type (normal or reactive) (41). For example, during the rat hippocampus development, the glutamate dehydrogenase activity in astrocytes increases, which might be due to the maturation of the glutamatergic structures (42). Also, it is important to consider that glutamate dehydrogenase activity increases simultaneously as that expression of GLT-1, which has been suggested as a regulatory system of glutamatergic dynamics (42). For the other two enzymes (aspartate aminotransferase and glutaminase), there is evidence of their presence in astrocytes (43), considering them for glutamate metabolism. To study the aspartate aminotransferase enzyme in astrocytes, L-[U-C^{14}] and L-[I-C^{14}]

glutamate have been used; as the main product of these compounds' metabolism is CO_2, the concentration of this gas was measured in hyamine hydroxide with a special chamber. Using this method, it was determined that an increase of CO_2 was observed in astrocytes primary cultures, suggesting that aminotransferase enzyme is present (44), and the enzyme glutaminase was determined using its inhibitor NEM (N-ethylmaleimide). In this case, the enzyme activity was evaluated according to different concentrations of NEM (45).

On the other hand, there is evidence that cultured astrocytes can synthesize glutamate using pyruvate carboxylase, a process that occurs in the cytosol (46).

Glutamate packaging.

After glutamate has been synthesized in neurons, it is packaged and stored in synaptic vesicles. To refill these synaptic vesicles with glutamate, it is necessary a vesicular glutamate transporter (VGLUT). There are three types of VGLUTs (VGLUT 1, 2, and 3), and they are widely distributed in the central nervous system. It was determined by immunocytochemistry that VGLUT 1 is present in the cerebral and cerebellar cortex, in the hippocampus, and the thalamus (47,48). VGLUT 2 is present in the cortex, thalamus, diencephalon, and rhombencephalon (48,49). VGLUT3 is less frequent than the other two, but it is located in the striatum, neocortex, and hippocampus (50).

There is evidence indicating that the three VGLUTs are also presented in astrocytes (50,51). Using immunocytochemical detection from rat brain preparation, VGLUT 3 presence was described in astrocyte end-feet (50). For VGLUT 1 and VGLUT 2, immunocytochemistry and nuclear staining were made in Sprague Dawley

brain rats from 0 to 2 days old. The results suggested VGLUT 1 and 2 in astrocytes, confirmed by immunoblots and RT-PCR (51). Similar experiments demonstrated VGLUT 1-3 in the mouse cortex, hippocampus, and cerebellum; these experiments were performed in culture and *in situ* astrocytes (52). The presence of these VGLUT's in astrocytes suggested that astrocytes can release vesicular-packaged glutamate, also known as gliotransmission.

One feature of the VGLUT uptake system is the dualism of the transporters with chloride ions (Cl^-). This ion has an important role in glutamate uptake according to the extravesicular concentration, in this case, there are three conditions: the first one consists of an insufficient extravesicular Cl^- concentration that generates high glutamate uptake, the second one corresponds to a high Cl^- concentration, which causes a gradual inhibition of glutamate uptake. The last one, an absence of Cl^-, generates a very low glutamate uptake (53,54).

Changes in the expression of the VGLUTS have been observed in neurodegenerative diseases. In Parkinson's disease, the expression of VGLUT1 and 2 increase in the putamen, and VGLUT 1 decreases in the prefrontal and temporal cortex (55). In Alzheimer's disease, VGLUT 1 and 2 decreases in the prefrontal cortex (56). These investigations were carried out in postmortem human brains using immunohistochemistry, immunoautoradiography, western blot, and choline acetyltransferase (ChAT) assay techniques.

Glutamate release.

Once glutamate is packaged into the synaptic vesicles and stored in the synaptic button, it is ready to be released once the action potential arrives. The action potential is the basic event that neurons use to transmit information to each other.

The action potential is composed of four phases: the rising phase known as depolarization (-70 mV to +40mV), the threshold, which is the value that the membrane potential needs to reach the action potential (the all or nothing response, -55mV), the repolarization phase, where the value of the membrane potential return to the resting potential (+40 mV to -70 mV), and the hyperpolarization (-70 mV to -90 mV), that is the phase at which the membrane potential can be more negative than the resting potential. The action potential is generated by the opening and closing of Na^+ and K^+ channels that increase and decrease the conductance of each ion in the different phases. Another feature of the action potential is its high efficiency to propagate itself through the axon until it reaches the synaptic button inducing the neurotransmitter release (57).

At the synaptic button, the arrival of the action potential induces the opening of voltage-dependent calcium channels, increasing intracellular calcium concentration, which will allow the fusion of the vesicle with the plasma membrane of the synaptic button and the release of the neurotransmitter (fig. 2) (58). The SNARE (Soluble NSF Attachment Proteins Receptor) are the main proteins involved in this process. These proteins are divided into two groups, the v-SNARE, v- is for vesicles, and these are highly expressed in vesicles, and the t-SNARE, t- is for target, and these are highly expressed in the target zone in the cellular membrane

(59). A trans-SNARE complex - interaction between v and t SNARE's- must be formed to release the neurotransmitter in either neurons or astrocytes. The main v-SNARE are synaptobrevin, syntaxin, and SNAP-25 for t-SNARE.

The presence of synaptobrevin 2 was confirmed in rat astrocytes by electron microscopy and calcium imaging, which suggests that Ca^{+} is the major contributor to gliotransmitter release (60). Also, astrocytes express syntaxin and SNAP-25 as detected by immunohistochemistry. Glutamate release from astrocytes was evaluated by potent inhibitors of neuronal exocytosis such as clostridial neurotoxins (ClTx), tetanus toxin (TeTx), and botulinum toxins (BoTx). In this case, BoTx-A (which cleaves SNAP-25) was used to determine SNAP-25 and BoTx-C (which inhibits syntaxin) for syntaxin, after the treatment with these toxins, a decrease in glutamate release was observed (61).

It is important to mention that the vesicular exocytosis process is similar between neurons and astrocytes (60), but the initial stimulus that induces vesicular release is slightly different. Since the astrocytes cannot generate action potentials, the rise in intracellular calcium is generated by activating ionotropic and metabotropic receptors (62,63). In this regard, ionotropic receptors, NMDA, AMPA, and KA receptors, belong to the glutamate receptor family (64–66) and P2X1-7 ATP receptors, also known as purinergic receptors, have been identified in astrocytes (67,68). The metabotropic receptors related to astrocyte increase in calcium are the glutamatergic mGluR3 and mGluR5 (62,69,70), and the P2Y13 purinergic receptors (68).

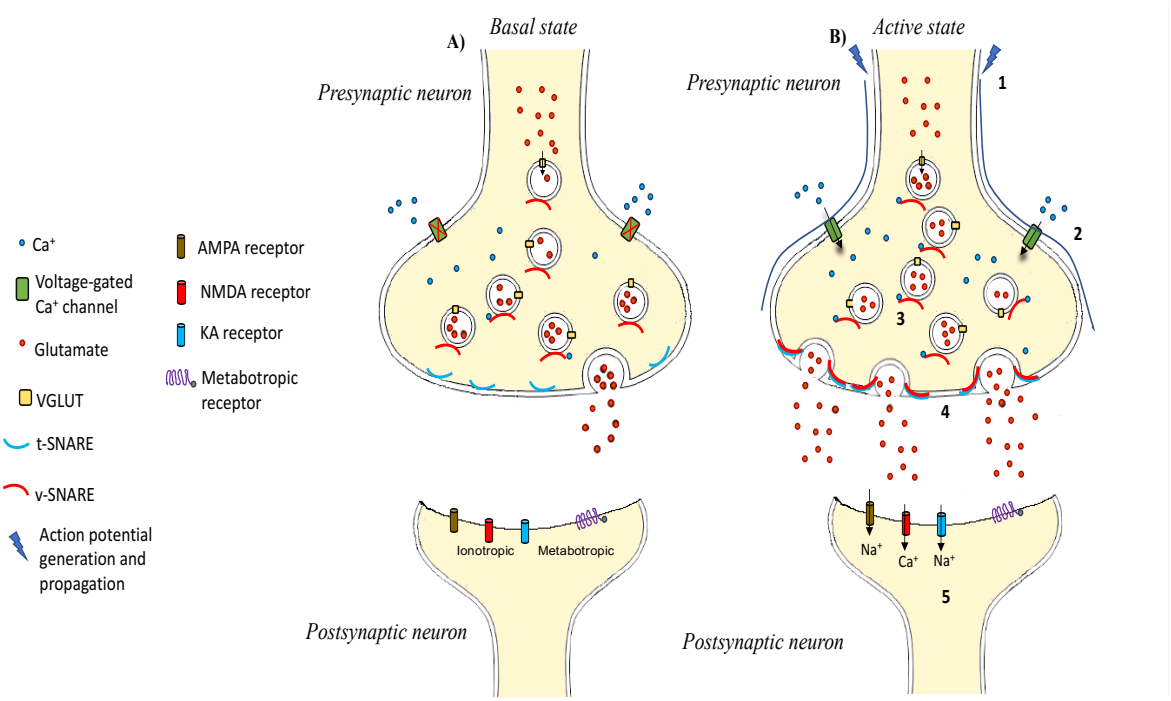


Fig. 2.- Glutamate release. - Schematic illustration of vesicular glutamate release from the presynaptic neuron during spontaneous release and active state. A) The basal or spontaneous. During the spontaneous activity, no action potential occurs, and voltage-dependent Ca^{+} channels remain closed, which keeps calcium concentration low, and there is no massive release of the neurotransmitter (glutamate). However, during spontaneous activity, a small amount of neurotransmitters is released into the synaptic space. B) During the active state, five steps for the massive glutamate release occur. 1) arrival of the action potential to the synaptic button. 2) The action potential generates a conformational change in the voltage-dependent Ca $^{+}$channels (closed-to-open), increasing the intracellular calcium concentration. 3) The Ca^{+} ions interact with the v-SNARE proteins, resulting in the movement of the vesicles to the t-SNARE proteins 4) to form the trans-SNARE complex and the consequent fusion of the vesicle in the synaptic button membrane

for the glutamate release. Finally, 5) the neurotransmitter activates the different types of receptors (AMPA, KA, NMDA, or metabotropic) present in the postsynaptic membrane (modified from Popoli et, al. 2012 (71)).

Glutamate receptors.

Receptors are transmembrane proteins located in neurons and glial cells, upon its binding, the neurotransmitters induce a conformational change that induces the activation of the receptor. Glutamate activates two types of receptors: ionotropic glutamate receptors (iGluRs) and metabotropic glutamate receptors (mGluRs). Next, a detailed description of each one will be provided.

Ionotropic glutamate receptors (iGluRs).

The iGluR receptors are responsible for the vast majority of excitatory transmission in the central nervous system of vertebrates, and these receptors are responsible for faster synaptic transmission. Ionotropic glutamate receptors are ligand-gated ion channels and allow the movement of cations such as Na^+, K^+, and Ca^+ across the cell membrane. iGluRs are transmembrane proteins composed of four subunits that form a central ionic pore; structurally, they have an extracellular amino-terminal domain, an extracellular ligand-binding domain, four transmembrane domains, and an intracellular carboxyl-terminal domain. In this group, we can find NMDA (N-methyl-D-aspartate), AMPA (α-amino-3-hydroxyl-5-methyl-4-isoxazol-propionate) and KA (kainic acid) receptors, named after the agonist that activates them. For these receptors to be functional, they must be assembled exclusively by the subunits

belonging to the same receptor class (72,73).

NMDA receptor.

NMDA receptors are distributed widely in the central nervous system and are widely studied in physiological and pathological mechanisms, mainly during excitotoxicity (74). The subunits that constitute this receptor are GluN1, GluN2A–GluN2D, GluN3A, and GluN3B; a functional receptor contains a tetrameric assembly by two GluN1 subunits together with either two GluN2 subunits, or a combination of GluN2 and GluN3 subunits (75,76).

Full activation of NMDA receptors occurs once glutamate and its co-agonist glycine bind to the receptor, which will allow the influx of Na^+ and Ca^+ ions after the membrane depolarization releases the magnesium ion that blocks the channel. Glycine binding sites are located at the GluN1 and GluN3 subunits. HEK 293 cells exposed to different concentrations of glycine and glutamate 1 mM, showed an increase in the activity of the NMDA receptor, since it has been shown that the NMDA receptor in these cells is conformed, in its majority, by the subunit GluN1 (77). For the GluN3 subunit, the expression was generated in oocytes and the electrophysiological records were made in the presence of a GluN1 and GluN3 glycine binding site antagonist, TK40. It was observed a decrease in the electrical activity of the oocytes expressing the GluN1 and GluN3 subunits (78). The GluN2 subunit contains the glutamate binding site as determined by crystallography (79). Altogether, these results indicate that glycine is an important co-agonist of NMDA receptors. Depending on the receptor's subunit composition, a differential activity might occur depending on glycine extracellular concentrations. Interestingly, glycine

and also D-serine are release by astrocytes (80,81).

Astrocytes also express NMDA receptors, as evidenced by the presence of GluN1 and GluN2 subunits mRNA (82). Moreover, there is a differential expression pattern of NMDA subunits in astrocytes that vary according to the brain regions. For example, cortical astrocytes express GluN1 and GluN2A/B subunits (83), also, in the same region, GluN2C and GluN2D are highly expressed (84). GluN2B is more abundant in the Bregman glia cells (85), while the GluN2C is predominantly expressed in the telencephalon glial cells (86). In the hippocampus, GluN1 and GluN2 subunits are present (87). The combinations of the NMDA receptor subunits are very important because depending on the subunit composition, NMDA receptors might have differential functional properties. Besides, some studies suggest that this receptor participates in the modulation of intracellular astrocytic Ca^+ concentrations for the gliotransmission process (88).

AMPA receptor.

AMPA receptors are composed of four subunits, GluA1, GluA2, GluA3, and GluA4, that can conform homomers and heteromers. In the nucleus accumbens, dorsal striatum, prefrontal cortex, and hippocampus have been reported the presence of these subunits, varying in number according to the region (89). This receptor allows the Na^+ and K^+ influx, but if the receptor conformation lacks a GluA2 subunit, Ca^+ will be permeable.

Some studies suggest the presence of AMPA receptors in glial cells. This receptor was described in cortical astrocytes through the use of electrophysiology and pharmacological agents (90). Similarly, this receptor was found in astrocytes

from the mouse hippocampus (91). In astrocytes from this region, the AMPA receptor modulates the inward-rectifier potassium channels (Kir), a rectifying inward current that passes easily through potassium channels. This current is also important for the regulation of neuronal functioning during AMPA biophysical stages (92): as a regulator of gliotransmitter release generated by Ca^+ signaling (93) and their participation in neurodegenerative disorders (94). To noteworthy, AMPA receptors expressed in astrocytes are permeable to Ca^+ because this receptor channel is formed by at least one GluA3 or GluA4 subunit (95).

The AMPA receptors are involved in cognitive processes such as learning and memory, as demonstrated with classic and novel optogenetic tools known as PhotonSABER. When PhotonSABER induced AMPA receptors endocytosis during active synapses, long-term depression becomes affected, which leads to altered learning and memory (96). Changes in AMPA receptors are also involved with chronic temporal lobe epilepsy, where a reduction in GluA1 and GluA2 subunits concentrations was reported (97).

KA receptor.

Among the iGluRs family proteins, KA receptors comprise the least studied class (98). Since the KA receptor belongs to the group of iGluRs, it will have a similar conformation to the other two receptors belonging to the group. The subunits that make up the KA receptor are GluK1, GluK2, GluK3, GluK4, and GluK5. While the subunits GluK1, GluK2, and GluK3 are considered low-affinity subunits, the GluK4 and GluK5 are high-affinity subunits (99,100). High-affinity subunits cannot form a

functional receptor, so they must interact with low-affinity subunits (101), forming a heteromeric receptor in a ratio of 2:2 subunits (102). Some of the most important functions of this receptor are the regulation of synaptic activity (103) and neuronal plasticity (104). However, alterations in KA receptors have been described in epilepsy (105) and schizophrenia (106,107).

The KA channel is permeable to ions such as Na^+ and K^+. However, when some modifications are generated in the subunits GluK1 and GluK2, it can also be permeable to Ca^{2+} (108). The expression of the different subunits of the receptor varies according to the region of the brain, for example, in the primate neocortex, the GluK1-2-3 subunits are mostly expressed (109). In the rodent cortex, a greater expression of GluK2 and GluK4 subunits occur (110).

It has been reported that perivascular astrocytic processes express the GluK1 subunit and mediates astrocytes-related glutamate response (111). Also, GluK1-3 subunits are present in astrocytes, specifically, in the hypothalamic arcuate nucleus. In this region, the main function of this receptor depends on an estrogen-glutamate pathway (66). In the hippocampus, particularly in CA1, all the KA subunits are expressed, and alterations in them are related to the status epilepticus. The expression of all KA subunits increases after the generation of the status epilepticus induced by kainite administration. Furthermore, it has been suggested that KA receptors could function as glutamate sensors (112).

Metabotropic receptors (mGluRs)

mGluRs are coupled to G-proteins and modulate slow synaptic transmission through second messengers. To date, 8 mGluRs (mGluR1-8) have been described, which

have been divided into three groups (I, II, and III) according to the characteristics they possess: similarity in their sequences, their pharmacological properties and the intracellular signaling mechanisms they trigger (113).

The structure of these receptors consists of an extracellular N-terminal domain, a cysteine-rich region, seven transmembrane domains, and an intracellular C-terminal domain. The N-terminal portion has the glutamate binding site ("flycatcher" conformation) and the C-terminal portion regulates the activity of the receptor (114).

The group I receptors, mGluR1 and mGluR5, are associated with intracellular calcium signals, phospholipase C, and are activated mainly by 3.5-DHPG. Group II includes mGluR2 and mGluR3, which are negatively coupled to adenylate cyclase and have a selective agonist, LY379268. Finally, the mGluR4, mGluR6, mGluR7 and mGluR8 receptors conform group III, which, like group 2, are negatively coupled to adenylate cyclase and are activated mainly by 2-amino-4-phosphonobutyrate (115,116).

As described in neurons, the expression of mGluRs in astrocytes varies according to the brain region. Hippocampal astrocytes isolated from rats showed a predominance of mGlur3, and mGlur5 receptors (117). Using electron microscopy and immunohistochemistry, mGluR2 and mGluR3 were identified in the rat ventrobasal thalamus (118).

In astrocytes, mGluR3 and mGluR5 activation generate Ca^{2+} signaling (62,69,70) that is related to the control of extracellular glutamate concentration by increasing the expression of glutamate transporter (GLAST) (119–121). This increase in expression was observed when an agonist of the metabotropic group II

receptors is used and the increase of GLAST expression was confirmed by western blot (121). There is evidence that the mGluR5 receptor also affects the functioning of the GLT-1 transporter (122).

Glutamate transporters.

Glutamate transporters also known as excitatory amino acid transporters (EAATs), maintain an optimal glutamate concentration in the synaptic cleft. These proteins are expressed in neurons and glial cells, especially astrocytes, responsible for the bulk of glutamate uptake (31). These transporters belong to the SLC1 family (high-affinity glutamate transporters) (123), and its function is based on the co-transport of glutamate, Na^+ (three molecules), H^+ (one molecule), and counter-transport of K^+ (one molecule) (124).

To understand the main function of these transporters, the structure was determined by crystallography (125). The structure indicated the presence of a scaffold domain and a transport domain, the first one is composed of transmembrane helices (TM 1-2 and TM 4-5), and the second is composed of TM 3, TM 6-8 and a re-entrant helical loop 1-2 (125–127). While the scaffold domains maintain the structure in the membrane by protein-protein association, the amino and carboxyl-terminal domains remain inside the cell (30). Five EAATs, in humans, have been identified, EAAT 1-5 corresponding to SLC family genes expression. In rodents, as a good model for the study of the nervous system, these transporters proteins were named as excitatory amino acid carrier 1 (EAAC1/EAAT3), the glutamate-aspartate transporter (GLAST/EAAT1), the glutamate transporter 1 (GLT-1/EAAT2), the

excitatory amino acid transporter 4 (EAAT4) and the excitatory amino acid transporter 5 (EAAT5) (fig. 3) (30–33,128,129). All of these transporters share a high percentage of amino acid sequence (130), but they differ in the cellular and developmental expression according to the brain regions (131,132), and alteration in their function contributes to neurodegenerative diseases and neuropathologies (133–135).

in situ hybridization and immunohistochemistry studies showed that neurons of the cerebral cortex, hippocampus, cerebellum and spinal cord express EAAT 3 (136,137). The EAAT 4 is also expressed in neurons as detected by immunohistochemistry and *in situ* hybridization, but its localization is mostly in the rat fore and midbrain (138). The EAAT 4 was identified in the mouse somatosensory cortex by immunohistochemistry (139). On the other hand, GLAST and GLT-1 are highly expressed in astrocytes (140,141). However, an isoform GLT 1b has been described in hippocampal neuronal terminals (142). The expression of GLT-1 is more abundant in the hippocampus, striatum and cerebral cortex (140,141,143,144). Also, GLAST transporter is highly expressed during neuronal development (42,145) and in the cerebellum (146–148). GLAST and GLT-1 have an important role in cerebral physiology because a change in their function or its number in the membrane might modify brain neuronal processing contributing to neuronal death in early development stages and/or neurodegeneration (145).

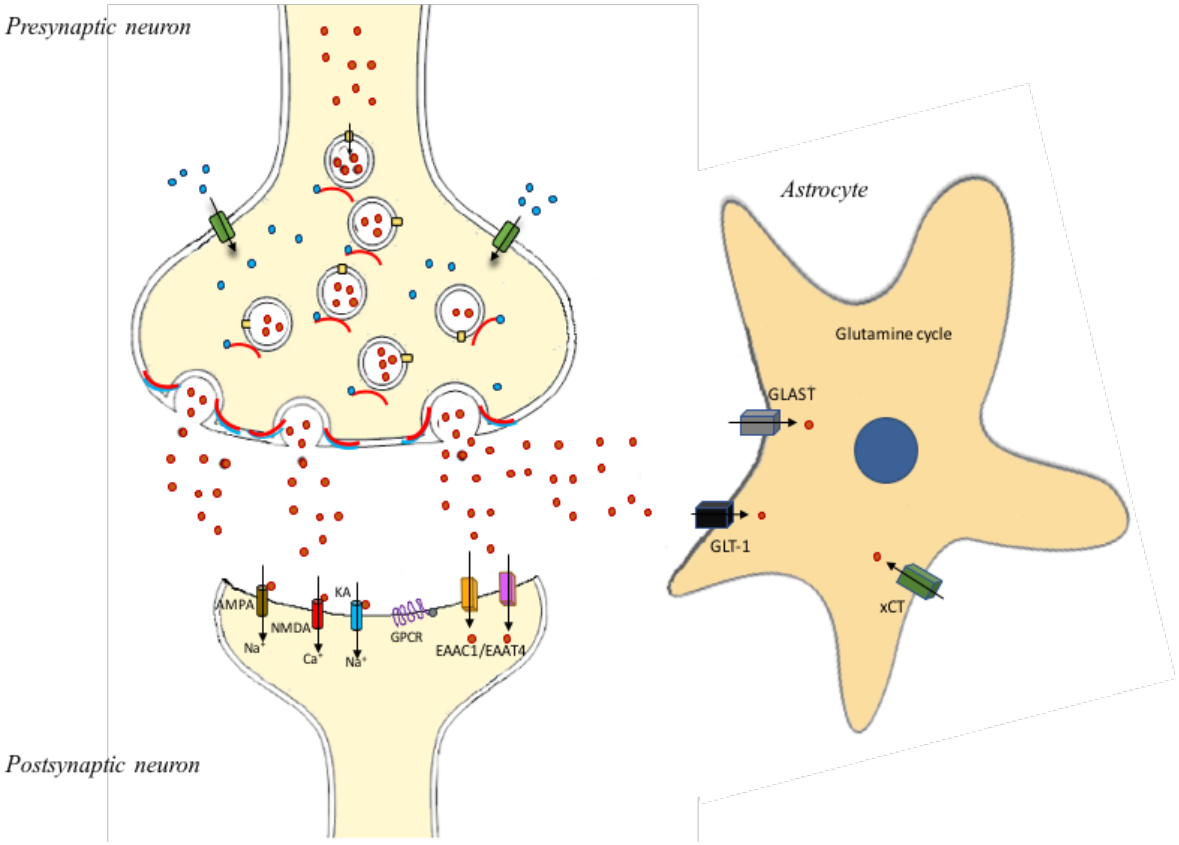


Fig. 3.- Glutamate transporters. - Distribution and function of glutamate transporters. After glutamate is released and its effects occur through its receptors, it will be recaptured by different transporters located in neurons (EAAC1 and EAAT4) and astrocytes (GLT-1, GLAST and xCT). Once astrocytes take up glutamate, the glutamine cycle will occur for the subsequent formation of glutamine (modified from Popoli et, al. 2012 and Estrada-Sánchez et, al. 2012 (71,149).

Some studies suggest that the same astrocyte can express both transporters. The first evidence was obtained when the expression of these transporters was characterized in three conditions, cultures of undifferentiated rat cortical astrocytes,

astrocytes cocultured with cortical neurons, and astrocyte cultures differentiated with dibutyryl cyclic AMP (dBcAMP). The final results indicated that the first condition expresses only GLAST transporter, the second one expresses both transporters, and the third one has an interesting pattern because the dBcAMP generates GLAST and GLT-1 expression. The expression of glutamate transporters was corroborated by Western blot, Northern blot, and immunohistochemistry (150). The same expression pattern occurred with hippocampal cocultures (151). On the other hand, a similar effect is observed with the brain endothelial cells. Astrocytes cocultured with endothelial cells, generate the expression of GLT-1 by the Notch signaling, a pathway implicated in *vitro* and *in vivo* endothelial-astrocyte communication (152). Also, there is *in vivo* evidence about the coexpression of these transporters; for example, a redistribution and function of both transporters were observed in the rat striatum in the presence of the astrocyte activator CNTF (ciliary neurotrophic factor) (153).

Moreover, glutamate transporters are involved in neuronal physiological properties, for example, the excitatory postsynaptic currents (EPSCs) of cortical pyramidal neurons are affected when GLT-1, GLAST, or both are blocked by the inhibitors DHK, UCPH-101, and DL-TBOA, respectively. The selective inhibition of individual transporters generates a decrease in the decay time of evoked EPSCs in the cortical pyramidal neurons, furthermore, the inhibition of both transporters generated a reduction of the EPSCs (154).

As mentioned above, alterations in the glutamate transporters are related to neurodegenerative diseases. Alzheimer's disease postmortem brains showed a

reduction in the EAAT1 and EAAT2 (155). In epilepsy, a decrease of EAAT 2 was reported (156). In Huntington's disease, reduced expression of GLT1 was observed in postmortem brain patients (157). Similarly, the R6/2 Huntington's disease transgenic mouse model showed a decrease in the content of GLT1 and GLAST, which correlates with increased vulnerability to glutamate-induced toxicity (20). Moreover, the R6/2 mice model presents altered cortical excitability when GLAST and GLT-1 are blocked (154). It is known that GLT-1 is predominantly more expressed than GLAST. This evidence has diverted the attention of the research groups towards the study of GLT-1 as the "master" transporter. However, what the other transporters can offer must be studied in-depth to understand better the relationships of all the cellular components present in the central nervous system.

Finally, the SLC711A transporter, unlike the previous ones, is a cysteine/glutamate antiporter, which will transport a cystine into the cell while exchanging for glutamate in a 1 to 1 ratio. Furthermore, this system is sodium-independent (158). Its structure consists of two subunits, the light subunit (SLC7A11) and the heavy subunit (SLC3A2). The light subunit comprises 12 transmembrane domains, an amino-terminal and a carboxyl-terminal domain arranged inside the cell. As for the heavy subunit, it is considered a unique transmembrane protein with an intracellular amino-terminal domain and a heavily glycosylated extracellular domain (159,160). It is important to mention that a disulfide bond links these two subunits and both subunits are essential for the optimal operation of the transporter. On the other hand, it has been possible to demonstrate that the light subunit is responsible for the active transport of cystine and glutamate. The heavy subunit is necessary to

assemble the light subunit in the membrane and structural stability (161,162). This system is important for glutathione biosynthesis, an essential cellular antioxidant, by reducing cystine to cysteine (main glutathione precursor) (163). Various evidence has managed to identify the presence of SLC7A11 in the brain (159). It has been shown that SLC7A11, in the brain, is essential for regulating glutamate and avoiding damage by oxidative stress (160). Although the transporter is highly expressed in the human brain (159), in various animal models such as mice, it is expressed in different proportions in different brain regions, such as the hippocampus, cortex, hypothalamus, and dentate gyrus (164). In addition to having managed to identify this transporter in different brain regions, with the use of antibodies, it was also possible to show that SLC7A11 is expressed mainly in glial cells (165). However, there is evidence that indicates greater expression in astrocytes (166). In addition, an increase in oxidative stress and astrocyte death was demonstrated by blocking SLC7A11 (167). This process is also known as oxidative glutamate toxicity (168).

Role of astrocytes in glutamatergic neurotransmission.

Some studies suggest that astrocytes can modulate directly or indirectly the synapses and neuronal physiology. For example, it has been suggested that astrocytes glutamate uptake it's a way to regulate the neurotransmitter levels in the synaptic cleft, which indirectly modulate neuronal transmission (15). Another mechanism might be the availability of glutamine to synthesize glutamate. In this regard, after glutamate is released and astrocytes have taken up the glutamate, it can be metabolized into α-ketoglutarate by glutamate dehydrogenase enzyme, or into glutamine by the amidation of glutamate to glutamine by the glutamine

synthetase enzyme (glutamine cycle) (35,169). As this enzyme is highly expressed in astrocytes, they are considered the major glutamine reservoir and an important part of the metabolism of the neurotransmitters like glutamate and GABA (170,171). Therefore, astrocytes might regulate glutamatergic neuronal dynamics by the amount of glutamine released into the synaptic cleft.

Another mechanism by which astrocytes can regulate neuronal activity is by releasing gliotransmitter, also known as gliotransmission. A gliotransmitter is a molecule that is stored and synthesized inside the glial cells, that is stored in vesicles and is released after a particular stimulation, which is highly regulated; once they are released, gliotransmitters are capable of activating responses (rapid/slow) in nearby cells, and these are involved in physiological or pathological processes (172). Through the use of electrophysiology, optical imaging and molecular biology techniques, it has been demonstrated that astrocytes are capable of responding to neurotransmitters and participate in the release of gliotransmitters such as ATP, glutamate, and D-serine (173–177). It is important to mention that gliotransmission has an important role in neuronal functioning, for example, through the release of ATP by astrocytes, an alteration in synaptic transmission can be observed (178). Recently, Shen et al., described that autocrine activation of P2Y1 purinergic receptors in astrocytes modulate the vesicular release of glutamate and the subsequent activation of extra synaptic NMDA receptors in neurons (179). Likely, this mechanism might also contribute to the development of some neurodegenerative diseases such as Alzheimer's, Huntington's disease and epilepsy (180–182)

In astrocytes, activation of metabotropic and/or ionotropic receptors generates an increase in Ca^{+} concentration, although it is known that metabotropic receptors contribute more than ionotropic (83,117). In terms of the low contribution of the ionotropic receptors, there are proves that demonstrate that some of them that are expressed in astrocytes have a special molecular structure conformation, in other words, these receptors include a subunit that reduces Ca^{+} permeability (65,88,111,183). For example, GluN3 subunits for NMDA (78,184), GluA2 for AMPA (185), and GluK3-4 for Kainate (108). This interesting pattern could be a regulatory system to avoid deregulations of the tripartite system.

After the neurotransmitter binds to metabotropic receptors, it causes responses associated with the activation of phospholipase C/inositol 1,4,5-trisphosphate (IP3) pathway, leading to IP3 formation that generates the release of Ca^{2+} from the endoplasmic reticulum (62,186) inducing the gliotransmitter release by Ca^{2+}-dependent exocytosis (60,187–190).

Since the metabotropic receptors are more effective in elevate Ca^{2+} concentration, it is important to consider that this process is slow due to their physiology. To understand more about this, the activation and the response generation time for the ionotropic receptor is approximately 1-10 milliseconds (185,191), and 50-200 milliseconds for metabotropic receptors (192,193).

Glutamate gliotransmitter modulates long-term depression in the hippocampus and cortex, modulated by the activation of NMDA receptors (194) (195). The activation of mGlur 1 and mGlur 5, in the striatum, generate a synaptic potentiation by increasing the excitatory postsynaptic currents (196); a similar effect is observed in the hippocampus, but in this case, the synaptic potentiation is also

favored by the effect of interneurons on the astrocytes through the increase of intracellular Ca^{2+} by the activation of $GABA_B$ receptor (197,198). In the cerebellum, activation of NMDA receptors increases the activity of inhibitory interneurons (199). Another example related to the activation of NMDA receptors, especially those with GluN2B subunit, is the Slow Inward Currents in the hippocampus, cortex (200,201) and nucleus accumbens (202). These currents are phasic extrasynaptic excitatory events with special characteristics, for example, the amplitude is 18-477 pA, the rise time comprises 13-332 decay times of 72-1630 milliseconds. It has been extensively demonstrated that these currents are generated by astrocyte activity. There are proves that exhibit a decrease of these currents if the Ca^{2+} signaling is abolished (203), also when intracellular Ca^{2+} concentration becomes greater, the slow inward currents increase (10,204). Altogether, this evidence indicated that metabotropic receptors are of great importance for neuron-astrocyte-synapse interactions (4).

There are distinct gliotransmitters release mechanisms; one of them is through hemichannels formed by connexins like Cx43, Cx30, and Cx36 (highly expressed in astrocytes) (205–207). There is evidence that the principal functioning mechanism is by altering the molecular structure of the hemichannel to make it permeable to another molecule, because, naturally, these hemichannels do not act as freely permeable non-selective pores (206). On the other hand, it is known that these connexins are involved in some diseases, for example, Alzheimer's disease, in this case, the connexin 43 network maintenance neuronal activity under amyloid β peptide (Aβ) stress, suggesting that connexin generate a neuroprotective response to amyloid plaques. Also, it regulates the Alzheimer's Disease risk genes expression (208–210). The Parkinson disease model (heterozygous Cx30 KO mice), showed

an increase in connexin 43 in the striatum and hippocampus, generating an elevation of the gap junctional intercellular communication, resulting in the disease development (211,212). In amyotrophic lateral sclerosis, similar to Parkinson disease, an increase of connexin 43 was reported, but in this case, this increase generates a release of reactive oxygen species and inflammatory mediators, also, the communication between astrocytes increased, causing an alteration of homeostatic processes such as small molecules and second messengers trafficking (213,214). During astrocytoma, connexins generate a stable network between cells that favors the increase of the tumor due to a shared metabolism (215). Moreover, there is evidence indicating that connexin 43 is necessary for glioma's motility, which was evaluated by reducing the levels of connexin 43 with RNAi in C6 cells. When comparing these cells with a high expression level, a greater movement was determined for those with more connexin 43. It is worth mentioning that motility was evaluated with the wound healing and transwell cell migration assays. On the other hand, it is emphasized in the connexin 43 carboxyl-terminus domain because this can interact with other proteins implicated in cell motility (216). Finally, it is important to mention that the molecular and pathophysiological mechanisms generated by connexins in all these diseases are unclear.

Schizophrenia

The study of mental disorders has been gaining relevance since the number of people suffering from them has increased considerably (217). The relevance of this type of disease relies on its impact on the patient and their family, due to

economic and social consequences. Currently, many mental disorders are characterized by abnormal social behaviors, irregular thoughts, and erratic emotional disturbances. These mental disorders include depression, bipolar disorder, autism, and schizophrenia. Despite a wide variety of studies that have been made on these disorders, their pathophysiology has not been fully understood.

Schizophrenia is characterized by sudden changes in behavior, alterations in perception, distortions in thinking, communication problems, and changes in cognitive understanding. Schizophrenia symptoms are divided into three categories: positive symptoms (hallucinations, delusions, disorganized behaviors, incoherence of speech, and erratic emotions), negative symptoms (social withdrawal and anhedonia), and cognitive symptoms (deficits in attention, working memory, verbal learning, and memory) (218,219).

Schizophrenia affects around 20 million people in the world, and some reports indicate that from all mental disorders, it is the least common (217). The diagnosis of this disease occurs particularly in late adolescence (17-19 years), and early adulthood (30 years). Also, it has been shown that this condition occurs earlier in men than in women. The mortality rate of these patients is much higher due to cardiovascular complications, metabolic diseases and suicide (220).

Regarding the neuropathology of schizophrenia, imaging studies revealed changes in brain structures such as an increase in the ventricles and a decrease in the volume of the prefrontal cortex and the hippocampus (221–223). In addition, there is evidence that indicates significant changes in the size and shape of neurons, which might alter their connectivity and synaptic activity (224). In addition to the brain morphology alterations, changes in the excitatory or inhibitory pathways contribute

to the development of schizophrenia (224). However, the relation between the structural alterations described and the neurotransmitters involved GABA, glutamate and dopamine in Schizophrenia neuropathology is still not completely understood (224,225).

Factors that might contribute to schizophrenia

Some risk factors that favor the development of schizophrenia have been established, such as genetic and epigenetic factors (226,227). According to a meta-analysis of the heritability of human traits in twins studies (228), genetic factors are the most predominant for the development of schizophrenia. However, epigenetic factors have become more relevant, since they have been increasingly identified (229).

The effects of the environment on pregnant women are one of the most important because everything the mother suffers will impact the development of the fetus. Obstetric complications are key to the development of schizophrenia, since they are related to an increase in the size of the cerebral ventricle and an increase in neonatal cerebrovascular events (230,231). Furthermore, prematurity, anoxia, infections during the pregnancy and prolonged labor are also related (232).

On the other hand, another factor that has become relevant today is fetal programming. The “origins in the development of health and disease” is a term that is defined as the process by which a stimulus or challenge establishes a permanent response. Some of the main factors involved in this process are genetic factors and the activation of genes by the pre-and post-natal environment. In addition, it has been established that the first thousand days of life (270 days from conception to

birth, 365 days equivalent to the first year, and 365 days equivalent to the second year) is a critical window of development since the factors that influence in this period will define the proper evolution of the organism (233)

The fetal programming of the offspring is considered as a physiological event in which the mother receives external stimuli that generate alterations in the embryo, that is, any stimulus (can be positive or negative) that reaches the mother, it will integrate it and affect the product under development (234,235). Currently, it has been possible to determine that pollution, physical activity, stress, environment, drugs, alcohol and infections are the main factors that affect the product under development, however, various studies have concluded that the main factor that generates alterations are that are related to diet. The fetal programming of the disease in adults derived from the mother's diet (caloric overfeeding), has generated some disorders such as behavioral modulation, obesity, coronary disorder, type 2 diabetes, and mental disorders such as schizophrenia (8,236,237).

Genetics factors.

Thanks to the new techniques and devices that allow the study of the genome, it has been possible to determine that many genes are involved in most diseases and neurodegenerative ones. Mutations in DNA have been considered the main factors in generating the transmission of schizophrenia through generations (238,239). However, it appears that schizophrenia is a multifactor disease.

To determine the impact of genes, comparative and integrative analyzes have been carried out in human brains and animal models. For example, a relationship

was reported between structural changes in the brain of patients suffering from schizophrenia and 32 polymorphisms of DNA, which are located in 30 genes related to neurogenesis and brain development (239). Moreover, it has been shown that the quaking gene (QKI) has an important relationship with the mRNA expression of six genes that are present in oligodendrocytes PLP1, MAG, MBP, TF, SOX10 and CDKN1B. This was determined by comparing brains from healthy subjects and subjects with schizophrenia; the real-time RT-PCR evaluation concluded a high proportion of expression of the genes mentioned above in healthy subjects. However, in samples from patients with schizophrenia, the mRNA expression levels of myelin-related genes (PLP1, MAG and TF) decreased significantly, around 68% to 96%. This evidence indicates that the gene QKI is essential for the optimal development of the oligodendrocytes, in addition, alterations in the functioning of the gene produce changes in the formation of myelin and, consequently, the generation of schizophrenia (240).

On the other hand, some hypotheses suggest that schizophrenia could be based on irregular neuronal migration, since the optimal functioning and development of brain structures depends on this, research has been focused on analyzing genes responsible for this process. For the generation of this study, a genotyping of SNP tags was performed in 18 genes involved in cytoskeletal adhesion and rearrangement. Thanks to the results obtained, it was possible to identify the MDGA1 gene as a candidate for susceptibility to the development of schizophrenia, which is involved in adhesion and migration processes (241).

Furthermore, the role of the gene NRG-1 has been studied extensively, as this plays a very important role in the development of schizophrenia (242). As for

neuregulins, proteins encoded by NRG-1, are proteins that exert their effect through erbB receptors, which are coupled to the tyrosine kinase response. It is important to mention that the functioning of this protein is highly related to the physiopathology of schizophrenia, since it could be related to the control of the expression of glutamate, $GABA_A$, and acetylcholine receptors (243–245). Finally, it has been shown that it is also responsible for the optimal development of oligodendrocytes (246).

As previously described, various genetic factors promote the development of schizophrenia, however, the basis for the development of this disease has not yet been established.

Role of glutamate in schizophrenia: Deciphering the origin of the disease.

As we can see, there are a wide variety of factors that might contribute to the development of schizophrenia. However, one component that has been consistently observed changes in the glutamatergic pathway. Nevertheless, it has not been possible to determine the full role of all glutamatergic components (receptors, transporters, and metabolism) on this disorder. To date, alterations in NMDA receptor activity, changes in the expression of glutamate transporters, and variations in vesicular glutamate transporters have been reported (247–249).

It has been possible to establish a hypofunction of NMDA receptors since, through antagonists, it was possible to mimic the phenotype of schizophrenia (247). Through the use of the anesthetic ketamine, a non-competitive NMDA receptor blocker, it was observed that at low doses (10, 20, and 30 mg/kg) increased

glutamate and dopamine levels. It is important to mention that this increase was registered in the prefrontal cortex and a rat model (250). Furthermore, ketamine has been used to generate models of schizophrenia in rats and mice (249–251). After noticing this pattern of glutamate release by blocking the NMDA receptor, it can be inferred that this increased release has consequent overstimulation of the other glutamate receptors, which might affect neuronal circuits functioning and might explain the altered pattern of behavior and cognition of schizophrenic patients (23,252). Regarding this glutamate dysregulation generated by ketamine, it should be considered as a coordinated event with other cellular structures and not as an isolated event, since, through correlation studies, it was possible to establish that mechanisms of calcium regulation through PMCA and postsynaptic density protein 95 (PSD95) are also involved in this process (249).

Another structure that is altered is the vesicular glutamate transporter (VGLUT). Using the Northern blot technique, it was possible to identify a decrease in VGLUT 1 mRNA in the hippocampal formation and the dorsolateral prefrontal cortex in the brains of patients with schizophrenia (253) . Moreover, a reduction in the expression levels of VGLUT-1 was observed in the rat depression model, suggesting, that this cellular component must be considered for the study of schizophrenia (254).

In addition, a reduction in complexin 1 and complexin 2 mRNA was also detected. The decrease in these proteins was observed in the superior temporal cortex, the dorsolateral prefrontal cortex (253).

As described above, the role of glutamate transporters plays an important role in maintaining optimal extracellular glutamate concentrations. Therefore, any

alteration that these suffer is important to consider. In this regard, *postmortem* brains of schizophrenia patients showed decreased glutamate transporters in astrocytes GLT-1 and GLAST in the superior temporal gyrus (255).

On the other hand, the decrease of GLT-1 and GLAST in the mediodorsal nucleus of the thalamus was also reported through the use of western blot. Furthermore, a compensatory pattern was observed, which consists of an increase in the expression of neuronal transporters in response to a decrease in the expression of transporters present in astrocytes (256). In addition, alterations have also been found at the post-translational level since lower glycosylation of the GLT-1 and GLAST transporters was identified. This study, like the previous ones, was carried out in human brains and was determined by western blot, however, it was detected by comparison of molecular weight (257,258).

The schizophrenia Ketamine model.

Evidence suggests that ketamine generates alterations in particular brain regions, such as the cortex, hippocampus, and midbrain (259–261). Also, some studies demonstrate that continuous administration of ketamine for fourteen days generates a phenotype similar to schizophrenia (262,263). As ketamine is an antagonist of NMDA receptors, we can explore how glutamatergic transmission is affected during this treatment. A reduction in the activity of these receptors by ketamine might induce a change in the presynaptic release of glutamate, which might affect the expression of glutamate transporters in neurons and astrocytes. Following this hypothesis, there is evidence indicating a decrease in the expression of the glutamate transporter GLT-1 and EAAT-3 due to the use of ketamine in the

hippocampus. However, the protocol on which they are based only refers to 5 continuous days of administration of ketamine, to mimic the depression phenotype. It is worth mentioning that these experiments were carried out in rats and the western blot technique was used to quantify the changes in the expression of the transporters (264).

Justification

As described above, alterations in glutamatergic transmission occur during schizophrenia. As the ketamine pharmacological model showed changes in glutamate release, it is possible that changes in the expression of glutamate transporters might take place, as they keep optimal extracellular glutamate concentrations avoiding harmful processes such as excitotoxicity. For this reason, it is important to explore the expression levels of the different glutamate transporters induced by ketamine.

Hypothesis.

Chronic treatment with ketamine generates an increase in the expression levels of GLAST and SLC7A11 / xCT transporters in the mouse hippocampus.

AIM.

To evaluate the effect of the chronic inhibition of NMDA receptors by ketamine, a pharmacological model of schizophrenia, on the expression levels of glutamate transporters in the mouse hippocampus.

Particular objective.

1. To measure the expression levels of the astrocytic transporter GLAST in the hippocampus after the chronic administration of ketamine.

2. To evaluate the expression levels of the transporter SLC7A/xCT in the hippocampus in response to the chronic inhibition of NMDA receptors by ketamine.

Chapter 1

Determination of the expression levels of the glutamate transporters GLAST and SLC7/xCT in the ketamine schizophrenia model.

Abstract.

The glutamate transporters play an important role in maintaining optimal extracellular glutamate concentrations, generating a correct neuronal function and avoiding harmful processes such as excitotoxicity. The analysis of these transporters is important since alterations in their functioning or expression levels have been reported in neurodegenerative diseases and mental disorders. Schizophrenia has been extensively studied to understand its pathophysiology and molecular mechanisms further and develop effective treatments. In the present work, we used the chronic inhibition of NMDA receptors by ketamine as a pharmacological model of schizophrenia to determine if this treatment alters the expression levels of the glutamate transporters (GLAST and SLC7A11/xCT). Our results indicated that while no changes in the expression level of GLAST occur, ketamine treatment increase the expression levels of SLC7A11/xCT expression in the hippocampus. As SLC7A11/xCT transporter contributes to the cysteine uptake to synthesize the antioxidant glutathione, an increase in the levels of this transporter might suggest that ketamine treatment might favor the production of oxidative stress. Further experiments are needed to explore this possibility and if ketamine alters other glutamate transporters.

Keywords: Glutamate, ketamine, schizophrenia, astrocytes.

Material and methods.

Ketamine model.

Mice were obtained from Círculo ADN SA de CV (Mexico City) and kept at the IPICYT under controlled humidity, temperature (22º C), with access to food and water *ad libitum* and 12h light/dark cycle. An assorted number of female and male mice were used. For the pharmacological model of schizophrenia, ketamine was intraperitoneally administered at a dose of 30 mg / Kg for 14 days, and control mice received an intraperitoneal injection of 0.9% sterile saline solution for 14 days. One day after the chronic treatment with ketamine, mice were anesthetized with sodium pentobarbital 10 mg / kg. After verifying the loss of sensitivity confirmed by a reflex scanning, the brain was removed and the hippocampus was dissected and kept at --80˚C until processing the sample to evaluate the changes in the expression of glutamate transporters by RT-qPCR.

For the experimental part of this work, a total of 3 mice in each group was used, the hippocampus for each hemisphere was processed separately.

RT-qPCR

The sample was frozen with liquid nitrogen and homogenized with the help of a potter's mortar. For total RNA extraction, TRIzol and phenol-chloroform were used. Subsequently, complementary deoxyribonucleic acid (cDNA) was synthesized from total RNA (1 µg). A volume of 20 µl was considered using a commercial cDNA reverse transcription kit (Thermo Scientific). A cDNA adjustment (500 ng / µL) was

performed. With the help of a CFX96 (Bio-rad) real-time system, real-time PCR was carried out. Finally, the reaction volumes were adjusted as follows: 2.5 µL of cDNA, 5 µL of Maxima SYBR Green Master Mix 2x (Thermo Scientific), 0.3 µL of each primer in a concentration of 10 µM, and 1.9 mL H20 DEPC. The primer sequences used for GLAST, SLCxCT, and βActin are shown in Table 2. The conditions of the RT-qPCR reaction were established according to the validation of the primers.

Results were obtained by normalizing each sample to its endogenous control (βActin gene). For the analysis, the $2^{-\Delta\Delta Ct}$ method was used, to obtain the relative expression of each mRNA relative to its control.

Primers design.

To determine changes in glutamate transporters expression, we design primers according to their nucleotide expression sequence. Using the Uniprot database, we found the glutamate transporters sequences, after that, we made a BLAST (tblastn) to generate the nucleotide sequence. Once the nucleotide sequence was obtained using ApE software, the primers were designed according to special features, such as Tm: 50-60º, G-C 40-60%, and an amplification range of 80-150 pb. Also, these primers were corroborated with idt dna software to avoid the formation of secondary structures (Table 1).

Table 1. Primers for the study of glutamate transporters.

	Primers			
Name	Sequence	Tm	G-C%	Amplification
GLAST	F-GCAGATGCCTTCCTGGATTT	58.23	50	127
	R-GTGTTTCGTTGGACTGGATAGG	59	50	
SLC7A11/xCT	F-CTTCGATACAAACGCCCAGATA	58.29	45.45	119
	R-CTGAATGGGTCCGAGTAAAGAG	58.21	50	
βActin	F-CAGCCTTCCTTCTTGGGTATG	58.35	52.38	99
	R-GGCATAGAGGTCTTTACGGATG	58.35	50	

Primer Validation.

To establish the optimal conditions for the RT-qPCR reaction and confirm that the amplification products only corresponded to the sequence of our interest, the validation of our primers was carried out. The validation data of βActin, GLAST and SLC711A/xCt is shown in the following graphs (Fig. 4, 5 and 6) through the amplification curve, melt curve, and melt peak. In addition, six different temperatures were tested to determine which one would have the best efficiency.

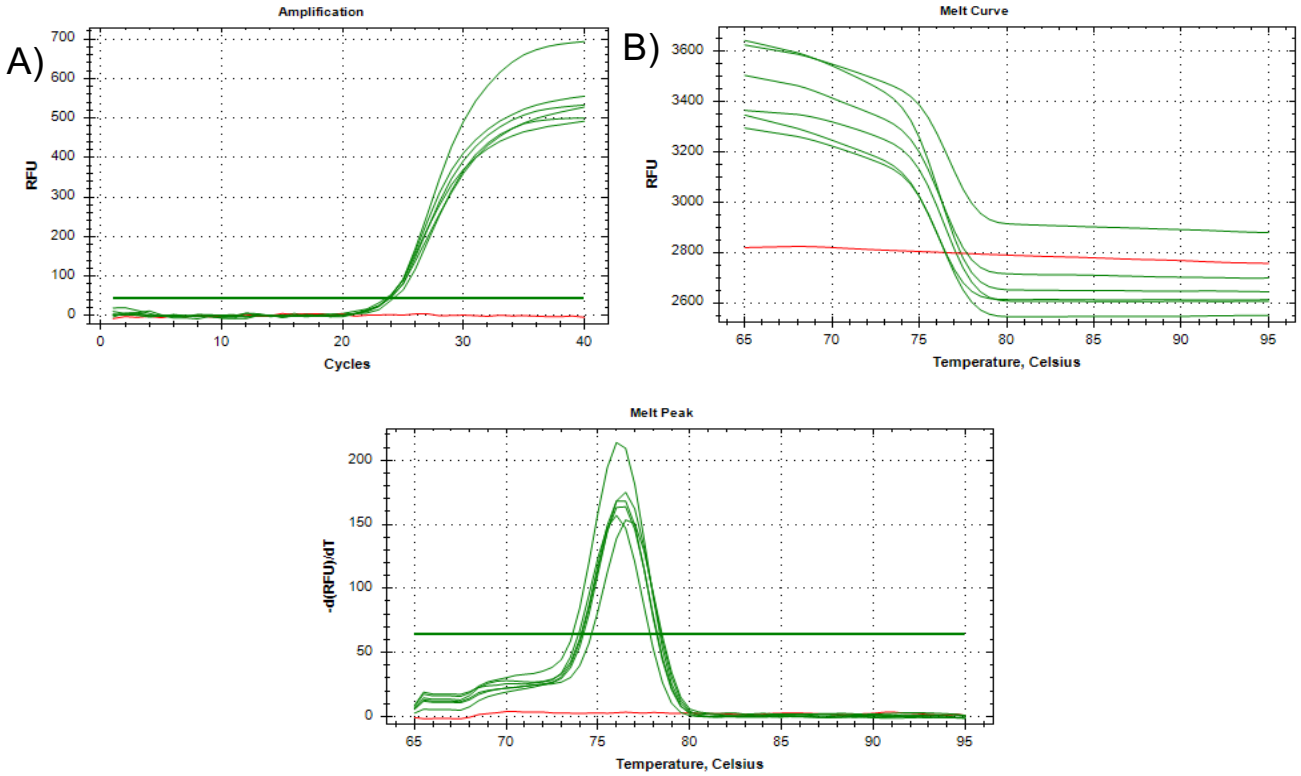


Fig. 4.- βActin primer validation. The following graphs show A) the amplification curve, B) melt curve and C) melt peak. For the generation of these curves, the following temperatures were used 56.7, 58, 60, 64.3, 65.5 and 66 ºC. The green curves represent the amplification, melt curve, and melt peak obtained for each temperature tested; the red curve is the NTC sample. The temperature of 56.7º C provided the best efficiency and was used for the experiments. The melt curve conditions were 65º C to 95º C (increment of 0.5º C for 0.05 sec).

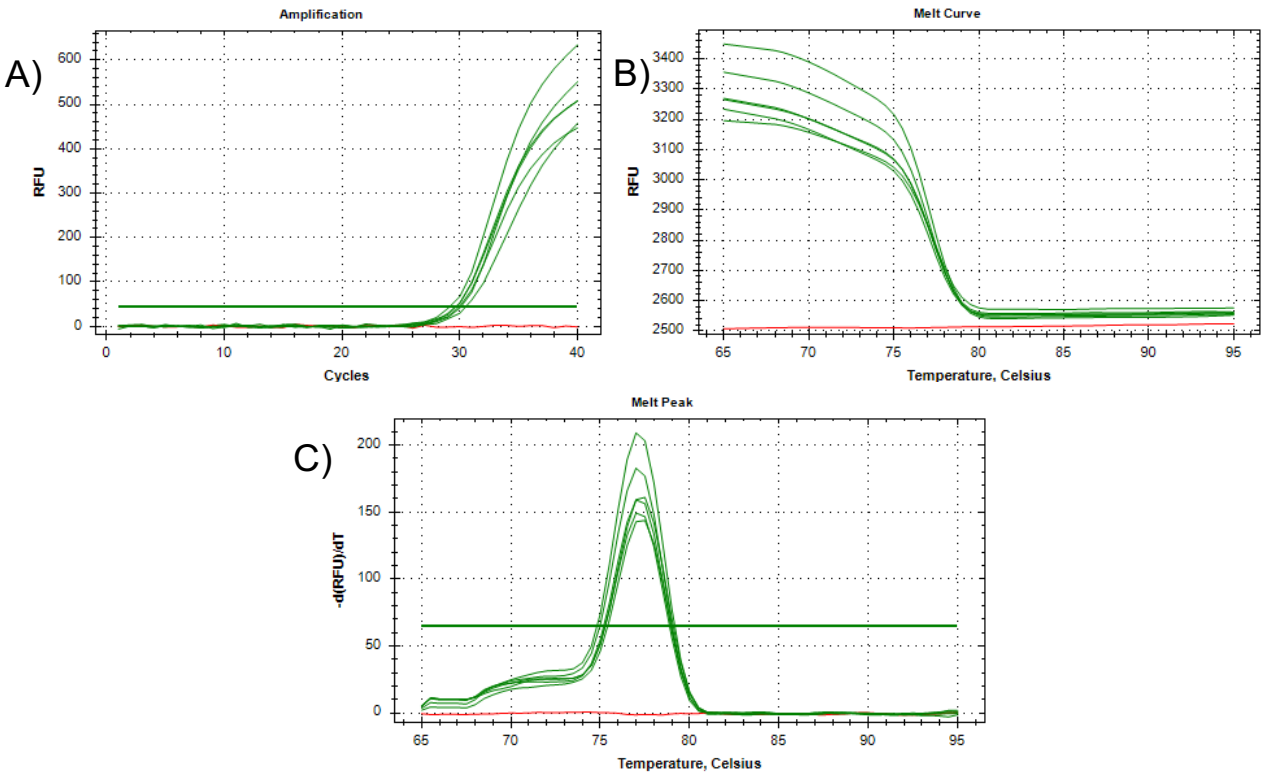


Fig. 5.- GLAST primer validation. The following graphs show A) the amplification curve, B) melt curve and C) melt peak. For the generation of these curves, the following temperatures were used 56.7, 58, 60, 64.3, 65.5 and 66 ºC. The green curves represent the amplification, melt curve and melt peak obtained for each temperature tested; the red curve is the NTC sample. The temperature of 56.7º C provided the best efficiency and was used for the experiments. The melt curve conditions were 65º C to 95º C (increment of 0.5º C for 0.05 sec).

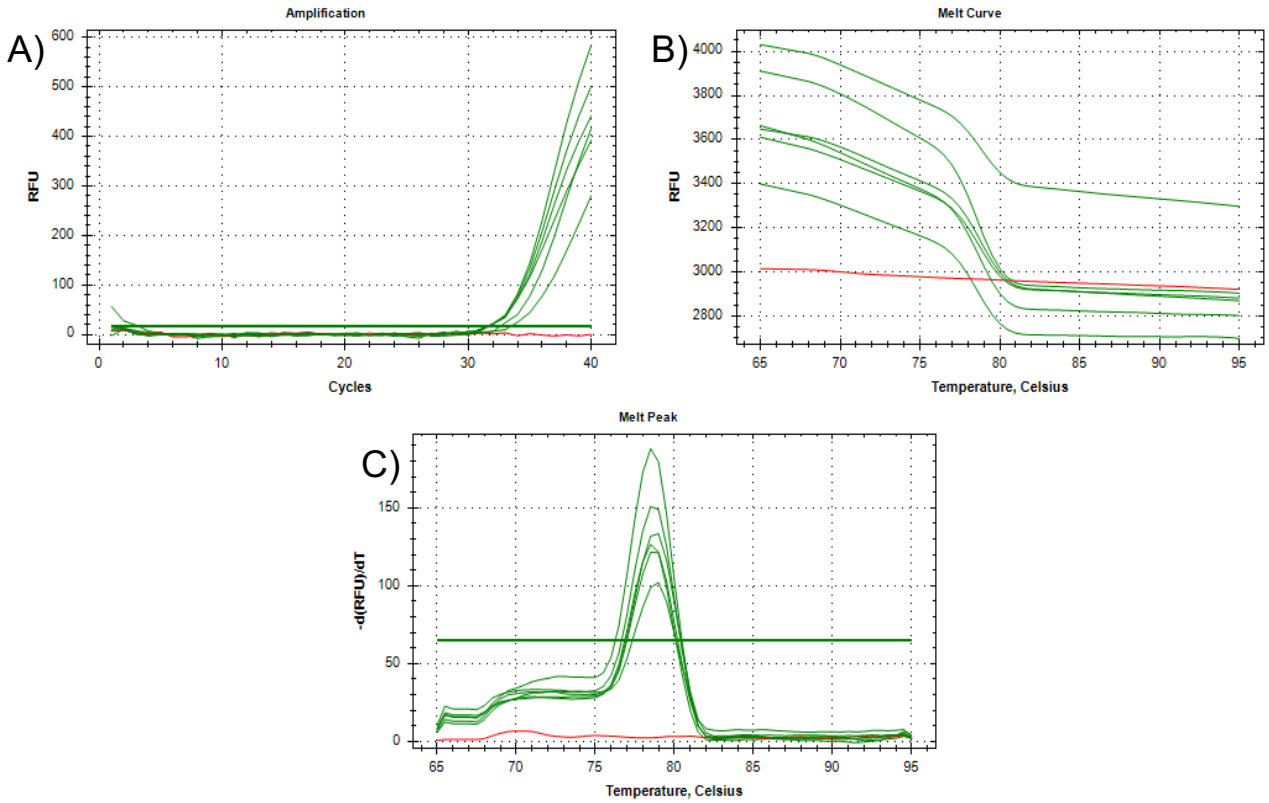


Fig. 6.- SLC711A/xCt primer validation. The following graphs show A) the amplification curve, B) melt curve and C) melt peak. For the generation of these curves, the following temperatures were used 56.7, 58, 60, 64.3, 65.5 and 66 ºC. The green curves represent the amplification, melt curve and melt peak obtained for each temperature tested; the red curve is the NTC sample. The temperature of 56.7º C provided the best efficiency and was used for the experiments. The melt curve conditions were 65º C to 95º C (increment of 0.5º C for 0.05 sec).

The number of reagents and biological material used for the validation of the primers was adjusted to a final volume of 10 µL of which 2.5 µL are cDNA, 5 µL of Maxima SYBR Green Master Mix 2x (Thermo Scientific), 0.3 µL of each primer (in a

concentration of 10 μM), and 1.9 mL H20 DEPC. The conditions of the RT-qPCR reaction were the following: pre-heating (95 ºC for 10 min.), denaturation (95 ºC for 15 sec.), primer annealing (56.7º C for 20 sec) and elongation (72.0º C for 40 sec), and 49 cycles were carried out.

Statistical analysis.

The data were analyzed using the Graph-Pad Prism program (GraphPad Software Inc., San Diego, CA). Initially, a normality test was carried out, and if the data were parametric, an unpaired t-test was performed. If data failed the normality test, then data were analyzed by a Mann-Whitney U test. All values were presented as mean ± SEM. A significant difference was considered when the p-value is <0.05.

Results and discussion.

Results.

As mentioned earlier, a decreased function of NMDA glutamate receptors might contribute to the development of schizophrenia, which perhaps might alter the levels of glutamate in the synaptic cleft (263), suggesting that changes in the glutamate transporters might also occur. According to the RT-qPCR analysis, similar levels of GLAST expression occur between the control group and group that received ketamine (30 mg / Kg for 14 days; Figure 4, left-hand side; p=0.6172). For the SLC7A11/xCT, the chronic inhibition of NMDA receptors induced a statistically significant increase in the expression of these transporters in the hippocampus (Figure 7, right-hand side; p=0.0458).

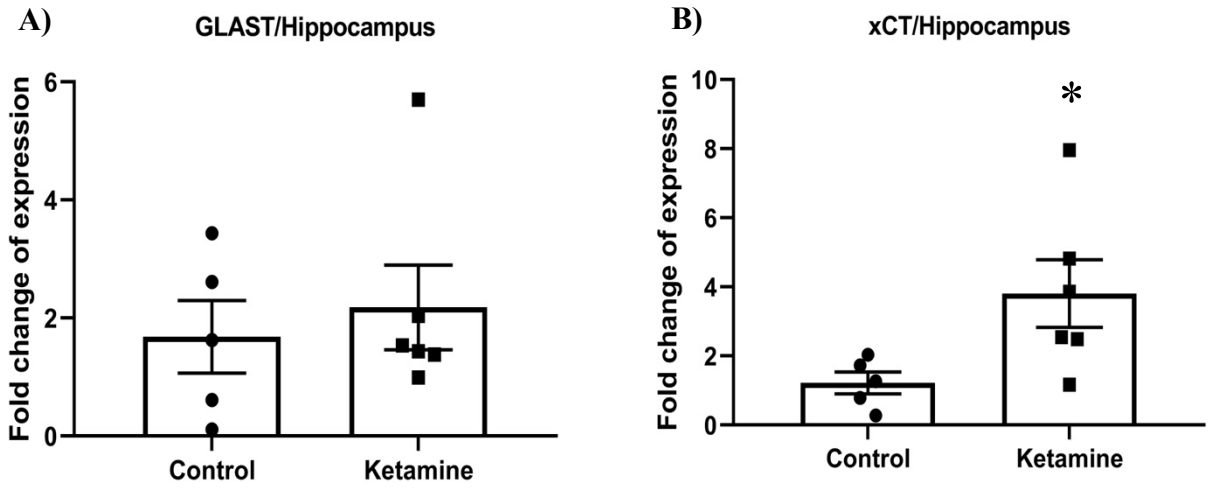


Fig. 7.- Expression levels of GLAST and SLC7A/xCT in the hippocampus after ketamine treatment. Changes in gene expression levels of the glutamate transporter GLAST (A) and the cysteine / glutamate transporter (B) are shown after

the administration of ketamine for 14 days. GLAST (p=0.6172) and SLC7A11/xCT (p= 0.0458). The significant difference between the control and ketamine groups is shown with an asterisk (* p-value is <0.05). In A, data were analyzed by Mann-Whitney U test. and in B, data were analyzed by unpaired t-test

Discussion.

It is now known that alterations in the glutamatergic pathway play a very important role in developing neurodegenerative diseases and mental disorders. This study analyzes the possible changes in the mRNA encoding the glutamate transporters GLAST and SLCA711 / xCT in the mice hippocampus after the pharmacological inhibition of NMDA receptors with ketamine to understand whether glutamate reuptake mechanisms are affected by the chronic inhibition of NMDA receptors. Our results showed that there are no significant changes in the expression levels of GLAST mRNA in the hippocampus, which suggests that astrocytic glutamate transporters might not contribute to the alterations elicited by the chronic ketamine treatment. However, to corroborate this possibility, we next should evaluate the changes in the GLT-1. Unfortunately, this was one of the aims of this project, but we had issues with the primer we developed. Nonetheless, this is an important component that needs to be further explored in different brain regions.

For the SLC7A11 / xCT transporters, we observed that chronic inhibition of NMDA receptors with ketamine-induced a significant increase in the expression of this transporter in the hippocampus. As SLC7A11 / xCT contributes to increasing glutathione synthesis through the co-transport of cystine by glutamate into the cell for subsequent metabolism, we could consider that treatment with ketamine could involve the generation of free radicals and that perhaps the increase of SLC7A11 / xCT expression could help protect against this damage. However, more studies are needed to explore this possibility.

According to the validation parameters of the primers for each gene, we obtained results that indicated a specific amplification of our sequence of interest. As amplification products were detected in the brain samples, we can conclude that all procedure was adequate.

This study focused on the hippocampus, but it should be considered that changes in glutamate transporters in other brain areas might occur after the ketamine administration. For example, in some regions of the temporal lobe of elderly patients with schizophrenia, there is a decrease in GLAST and GLT-1 (255).

Perhaps, glutamate transporters from other brain regions are more vulnerable to ketamine-induced changes. Furthermore, it was reported that higher doses of ketamine (50 mg / kg) reduced the expression of glutamate transporters in the hippocampus (265), which might also be a factor to consider for future experiments.

Conclusions.

Glutamate is the main excitatory neurotransmitter and it has a very important role in regulating various neuronal processes, and failure in the functioning of its components contributes to the development of neurodegenerative diseases and/or mental disorders. Given our current results, we observed that the chronic treatment with ketamine increased the expression levels of the SLC711A / xCT transporter. As SLC7A11 / xCT contributes to increasing the synthesis of the antioxidant glutathione through the co-transport of cystine by glutamate, our results suggest that ketamine treatment might lead to increased free radical production and possibly oxidative damage. In this sense, the increased expression of SLC7A11 / xCT might be a possible protection system. On the other hand, no change in the expression levels of GLAST was observed. However, it would be important to consider studying other transporters, for example, GLT-1 and EAAT-3, to understand the impact of ketamine on the glutamate reuptake system.

References.

1. Estacio-Gómez A, Hassan A, Walmsley E, Lee L, Southall TD. Dynamic neurotransmitter specific transcription factor expression profiles during Drosophila development. bioRxiv. 2019;830315.
2. Ventura R, Harris KM. Three-dimensional relationships between hippocampal synapses and astrocytes. J Neurosci. 1999;19(16):6897–906.
3. Allen NJ, Eroglu C. Cell Biology of Astrocyte-Synapse Interactions. Neuron [Internet]. 2017;96(3):697–708. Available from: https://doi.org/10.1016/j.neuron.2017.09.056
4. Araque A, Parpura V, Sanzgiri RP, Haydon PG. Tripartite synapses: Glia, the unacknowledged partner. Trends Neurosci. 1999;22(5):208–15.
5. Newman EA, Zahs KR. Modulation of neuronal activity by glial cells in the retina. J Neurosci. 1998;18(11):4022–8.
6. Simard M, Arcuino G, Takano T, Liu QS, Nedergaard M. Signaling at the gliovascular interface. J Neurosci. 2003;23(27):9254–62.
7. Giaume C, Tabernero A, Medina J. Metabolic Trafficking Through Astrocytic Gap Junctions. 1997;123(November 1996):114–23.
8. Maldonado-ruiz R, Marcela C, Montalvo-mart L, Vidaltamayo R, Garza-ocañas L, Res D. Priming of Hypothalamic Ghrelin Signaling and Microglia Activation Exacerbate Feeding in Rats ' O ff spring Following Maternal Overnutrition. 2019;
9. Smith M, Pereda AE. Chemical synaptic activity modulates nearby electrical synapses. Proc Natl Acad Sci U S A. 2003;100(8):4849–54.
10. Perea G, Yang A, Boyden ES, Sur M. Optogenetic astrocyte activation modulates response selectivity of visual cortex neurons in vivo. Nat Commun [Internet]. 2014;5:1–12. Available from: http://dx.doi.org/10.1038/ncomms4262
11. Behar TN, Scott CA, Greene CL, Wen X, Smith S V., Maric D, et al. Glutamate acting at NMDA receptors stimulates embryonic cortical neuronal migration. J Neurosci. 1999;19(11):4449–61.
12. Yu ACH, Hertz E, Hertz L. Alterations in Uptake and Release Rates for

GABA, Glutamate, and Glutamine During Biochemical Maturation of Highly Purified Cultures of Cerebral Cortical Neurons, a GABAergic Preparation. J Neurochem. 1984;42(4):951–60.

13. Hrabetova S, Serrano P, Blace N, Tse HW, Skifter DA, Jane DE, et al. Distinct NMDA receptor subpopulations contribute to long-term potentiation and long-term depression induction. J Neurosci. 2000;20(12):2–7.
14. Balcar VJ, Johnston GAR. THE STRUCTURAL SPECIFICITY OF THE HIGH AFFINITY UPTAKE OF l-GLUTAMATE AND l-ASPARTATE BY RAT BRAIN SLICES. J Neurochem. 1972;19(11):2657–66.
15. Jabaudon D, Shimamoto K, Yasuda-Kamatani Y, Scanziani M, Gähwiler BH, Gerber U. Inhibition of uptake unmasks rapid extracellular turnover of glutamate of nonvesicular origin. Proc Natl Acad Sci U S A. 1999;96(15):8733–8.
16. Bakaeva Z V., Surin AM, Lizunova N V., Zgodova AE, Krasilnikova IA, Fisenko AP, et al. Neuroprotective Potential of Peptides HFRWPGP (ACTH6–9PGP), KKRRPGP, and PyrRP in Cultured Cortical Neurons at Glutamate Excitotoxicity. Dokl Biochem Biophys. 2020;491(1):62–6.
17. Lucas, D.R., and NewHouse JP. The toxic Effect of sodium L-Glutamate on the inner layers of the retina. Arch Ophthalmol. 1957;58(2):193.201.
18. Valente P, Kiryushko D, Sacchetti S, Machado P, Cobley CM, Mangini V, et al. Conopeptide-Functionalized Nanoparticles Selectively Antagonize Extrasynaptic N‐Methyl‐. 2020;
19. Morrison LD, Becker L, Ang LC, Kish SJ. Polyamines in Human Brain: Regional Distribution and Influence of Aging. J Neurochem. 1995;65(2):636–42.
20. Estrada-Sánchez AM, Montiel T, Segovia J, Massieu L. Glutamate toxicity in the striatum of the R6/2 Huntington's disease transgenic mice is age-dependent and correlates with decreased levels of glutamate transporters. Neurobiol Dis [Internet]. 2009;34(1):78–86. Available from: http://dx.doi.org/10.1016/j.nbd.2008.12.017
21. Ahmed I, Bose SK, Pavese N, Ramlackhansingh A, Turkheimer F, Hotton G,

et al. Glutamate NMDA receptor dysregulation in Parkinson's disease with dyskinesias. Brain. 2011;134(4):979–86.

22. Donovan SMO, Sullivan CR, Mccullumsmith RE. The role of glutamate transporters in the pathophysiology of neuropsychiatric disorders. npj Schizophr. 2017;(August).
23. Olney J, Nuri B. Glutamate Receptor Dysfunction and Schizophrenia. Gen Psychiatry. 1995;
24. Zhou Y, Danbolt NC. Glutamate as a neurotransmitter in the healthy brain. J Neural Transm. 2014;121(8):799–817.
25. Niswender CM, Conn PJ. Metabotropic Glutamate Receptors: Physiology, Pharmacology, and Disease. Annu Rev Pharmacol Toxicol. 2010;50(1):295–322.
26. Zhang Z, Zhang S, Fu P, Zhang Z, Lin K, Ko JKS, et al. Roles of glutamate receptors in Parkinson's disease. Int J Mol Sci. 2019;20(18):1–17.
27. Tiburcio-Félix R, Escalante-López M, López-Bayghen B, Martínez D, Hernández-Kelly LC, Zinker S, et al. Glutamate-Dependent Translational Control of Glutamine Synthetase in Bergmann Glia Cells. Mol Neurobiol. 2018;55(6):5202–9.
28. Colbert CM, Levy WB. Electrophysiological and pharmacological characterization of perforant path synapses in CA1: Mediation by glutamate receptors. J Neurophysiol. 1992;68(1):1–8.
29. Boss V, Conn PJ. Metabotropic Excitatory Amino Acid Receptor Activation Stimulates Phospholipase D in Hippocampal Slices. J Neurochem. 1992;59(6):2340–3.
30. Hediger YK and MA. Primary structure and functional characterization of a high-affinity glutamate transporter. Nature. 1992;360:467–71.
31. Rothstein JD, Dykes-Hoberg M, Pardo CA, Bristol LA, Jin L, Kuncl RW, et al. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. Neuron. 1996;16(3):675–86.
32. Pines G. Cloning and expression of a rat brain L-glutamate transporter.

Nature. 1992;360:464–7.
33. Storck T, Schulte S, Hofmann K, Stoffel W. Structure, expression, and functional analysis of a Na+-dependent glutamate/aspartate transporter from rat brain. Proc Natl Acad Sci U S A. 1992;89(22):10955–9.
34. Hawkins RA. The blood-brain barrier and glutamate. Am J Clin Nutr. 2009;90(3).
35. Islam MM, Nautiyal M, Wynn RM, Mobley JA, Chuang DT, Hutson SM. Branched-chain amino acid metabolon: Interaction of glutamate dehydrogenase with the mitochondrial branched-chain aminotransferase (BCATm). J Biol Chem. 2010;285(1):265–76.
36. Plaitakis A, Nicklas WJ, Desnick RJ. Glutamate dehydrogenase deficiency in three patients with spinocerebellar syndrome. Ann Neurol. 1980;7(4):297–303.
37. Desai SN, Desai P V. Aspartate aminotransferase and alanine aminotransferase activities of rat brain during crush syndrome. Neurosci Lett. 2008;447(1):58–61.
38. Baglietto-Vargas D, López-Téllez JF, Moreno-González I, Gutiérrez A, Aledo JC. Segregation of two glutaminase isoforms in islets of Langerhans. Biochem J. 2004;381(2):483–7.
39. Yamada D, Kawabe K, Tosa I, Tsukamoto S, Nakazato R, Kou M, et al. Inhibition of the glutamine transporter SNAT1 confers neuroprotection in mice by modulating the mTOR-autophagy system. Commun Biol [Internet]. 2019;2(1):1–11. Available from: http://dx.doi.org/10.1038/s42003-019-0582-4
40. Masson J, Darmon M, Conjard A, Chuhma N, Ropert N, Thoby-Brisson M, et al. Mice lacking brain/kidney phosphate-activated glutaminase have impaired glutamatergic synaptic transmission, altered breathing, disorganized goal-directed behavior and die shortly after birth. J Neurosci. 2006;26(17):4660–71.
41. Osterberg KA, Wattenberg LW. Oxidative Histochemistry of Reactive Astrocytes. Arch Neurol. 1962;7(3):211–8.
42. Kugler P, Schleyer V. Developmental expression of glutamate transporters

and glutamate dehydrogenase in astrocytes of the postnatal rat hippocampus. Hippocampus. 2004;14(8):975–85.

43. Schousboe A, Svenneby G, Hertz L. Uptake and Metabolism of Glutamate in Astrocytes Cultured From Dissociated Mouse Brain Hemispheres. J Neurochem. 1977;29(6):999–1005.
44. Yu AC, Schousboec A, Hertz L. Metabolic Fate of 14C-Labeled Glutamate in Astrocytes in Primary Cultures. J Neurochem. 1982;39(4):954–60.
45. Kvamme E, Svenneby G, Hertz L, Schousboe A. Properties of phosphate activated glutaminase in astrocytes cultured from mouse brain. Neurochem Res. 1982;7(6):761–70.
46. Gamberino WC, Berkich DA, Lynch CJ, Xu B, LaNoue KF. Role of Pyruvate Carboxylase in Facilitation of Synthesis of Glutamate and Glutamine in Cultured Astrocytes. J Neurochem. 2002;69(6):2312–25.
47. Fujiyama F. TF and TK. Immunocytochemical localization of candidates for vesicular glutamate transporters in the rat cerebral cortex. J Comp Neurol. 2001;435(3):379–87.
48. Herzog E, Gilchrist J, Gras C, Muzerelle A, Ravassard P, Giros B, et al. Localization of VGLUT3, the vesicular glutamate transporter type 3, in the rat brain. Neuroscience. 2004;123(4):983–1002.
49. Fremeau RT, Troyer MD, Pahner I, Nygaard GO, Tran CH, Reimer RJ, et al. The expression of vesicular glutamate transporters defines two classes of excitatory synapse. Neuron. 2001;31(2):247–60.
50. Fremeau RT, Burman J, Qureshi T, Tran CH, Proctor J, Johnson J, et al. The identification of vesicular glutamate transporter 3 suggests novel modes of signaling by glutamate. Proc Natl Acad Sci U S A. 2002;99(22):14488–93.
51. Montana V, Ni Y, Sunjara V, Hua X, Parpura V. Vesicular Glutamate Transporter-Dependent Glutamate Release from Astrocytes. J Neurosci. 2004;24(11):2633–42.
52. Li D, Hérault K, Silm K, Evrard A, Wojcik S, Oheim M, et al. Lack of evidence for vesicular glutamate transporter expression in mouse astrocytes. J Neurosci. 2013;33(10):4434–55.

53. Wolosker H, De Souza DO, De Meis L. Regulation of glutamate transport into synaptic vesicles by chloride and proton gradient. J Biol Chem. 1996;271(20):11726–31.
54. Juge N, Yoshida Y, Yatsushiro S, Omote H, Moriyama Y. Vesicular glutamate transporter contains two independent transport machineries. J Biol Chem. 2006;281(51):39499–506.
55. Kashani A, Betancur C, Giros B, Hirsch E, Mestikawy S El. Altered expression of vesicular glutamate transporters VGLUT1 and VGLUT2 in Parkinson disease. Neurobiol Aging. 2007;28(4):568–78.
56. Kashani A, Lepicard È, Poirel O, Videau C, David JP, Fallet-Bianco C, et al. Loss of VGLUT1 and VGLUT2 in the prefrontal cortex is correlated with cognitive decline in Alzheimer disease. Neurobiol Aging. 2008;29(11):1619–30.
57. Hodkin, A. Huxley A. A QUANTITATIVE DESCRIPTION OF MEMBRANE CURRENT AND ITS APPLICATION TO CONDUCTION AND EXCITATION IN NERVE. J Physiol. 1952;177:500–44.
58. de Wit H, Walter AM, Milosevic I, Gulyás-Kovács A, Riedel D, Sørensen JB, et al. Synaptotagmin-1 Docks Secretory Vesicles to Syntaxin-1/SNAP-25 Acceptor Complexes. Cell. 2009;138(5):935–46.
59. Han J, Pluhackova K, Böckmann RA. The multifaceted role of SNARE proteins in membrane fusion. Front Physiol. 2017;8(JAN).
60. Crippa D, Schenk U, Francolini M, Rosa P, Verderio C, Zonta M, et al. Synaptobrevin2-expressing vesicles in rat astrocytes: Insights into molecular characterization, dynamics and exocytosis. J Physiol. 2006;570(3):567–82.
61. Jeftinija SD, Jeftinija K V., Stefanovic G. Cultured astrocytes express proteins involved in vesicular glutamate release. Brain Res. 1997;750(1–2):41–7.
62. Rodríguez-Prados M, Rojo-Ruiz J, García-Sancho J, Alonso MT. Direct monitoring of ER Ca2+ dynamics reveals that Ca2+ entry induces ER-Ca2+ release in astrocytes. Pflugers Arch Eur J Physiol. 2020;472(4):439–48.
63. Carmignoto G, Pasti L, Pozzan T. On the role of voltage-dependent calcium

channels in calcium signaling of astrocytes in situ. J Neurosci. 1998;18(12):4637–45.

64. Lee M, Ting KK, Adams S, Brew BJ, Chung R, Guillemin GJ. Characterisation of the Expression of NMDA Receptors in Human Astrocytes. 2010;5(11):1–11.
65. Seifert G, Rehn L, Weber M, Steinhäuser C. AMPA receptor subunits expressed by single astrocytes in the juvenile mouse hippocampus. Mol Brain Res. 1997;47(1–2):286–94.
66. Diano S, Naftolin F, Horvath TL. Kainate glutamate receptors (GluR5-7) in the rat arcuate nucleus: Relationship to tanycytes, astrocytes, neurons and gonadal steroid receptors. J Neuroendocrinol. 1998;10(4):239–47.
67. Kukley M, Barden JA, Steinha C. Distribution of P2X Receptors on Astrocytes in Juvenile Rat. 2001;21(June):11–21.
68. Carrasquero L, Delicado E, Bustillo D, Martin Y, Artalejo A, Portugal T. P2X7 and P2Y13 purinergic receptors mediate intracellular calcium responses to BzATP in rat cerebellar astrocytes. J Neurochem. 2009;879–89.
69. Cavaccini A, Durkee C, Kofuji P, Tonini R, Araque A. Astrocyte signaling gates long-term depression at corticostriatal synapses of the direct pathway. J Neurosci. 2020;(May).
70. Agulhon C, Sun MY, Murphy T, Myers T, Lauderdale K, Fiacco TA. Calcium signaling and gliotransmission in normal vs. Reactive astrocytes. Front Pharmacol. 2012;3 JUL(July):1–16.
71. Popoli M, Yan Z, McEwen BS, Sanacora G. The stressed synapse: The impact of stress and glucocorticoids on glutamate transmission. Nat Rev Neurosci [Internet]. 2012;13(1):22–37. Available from: http://dx.doi.org/10.1038/nrn3138
72. Ayalon G, Stern-Bach Y. Functional assembly of AMPA and kainate receptors is mediated by several discrete protein-protein interactions. Neuron. 2001;31(1):103–13.
73. Ayalon G, Segev E, Elgavish S, Stern-Bach Y. Two regions in the N-terminal domain of ionotropic glutamate receptor 3 form the subunit oligomerization

interfaces that control subtype-specific receptor assembly. J Biol Chem. 2005;280(15):15053–60.

74. Zhou X, Hollern D, Liao J, Andrechek E, Wang H. NMDA receptor-mediated excitotoxicity depends on the coactivation of synaptic and extrasynaptic receptors. Cell Death Dis. 2013;4(3):1–11.
75. Schorge S, Elenes S, Colquhoun D. Maximum likelihood fitting of single channel NMDA activity with a mechanism composed of independent dimers of subunits. J Physiol. 2005;569(2):395–418.
76. Ulbrich MH, Isacoff EY. Rules of engagement for NMDA receptor subunits. Proc Natl Acad Sci U S A. 2008;105(37):14163–8.
77. Cummings KA, Popescu GK. Glycine-dependent activation of NMDA receptors. J Gen Physiol. 2015;145(6):513–27.
78. Kvist T, Steffensen TB, Greenwood JR, Tabrizi FM, Hansen KB, Gajhede M, et al. Crystal structure and pharmacological characterization of a novel N-methyl-D-aspartate (NMDA) receptor antagonist at the GluN1 glycine binding site. J Biol Chem. 2013;288(46):33124–35.
79. Yao Y, Belcher J, Berger AJ, Mayer ML, Lau AY. Conformational analysis of NMDA receptor GluN1, GluN2, and GluN3 ligand-binding domains reveals subtype-specific characteristics. Structure [Internet]. 2013;21(10):1788–99. Available from: http://dx.doi.org/10.1016/j.str.2013.07.011
80. Neame S, Safory H, Radzishevsky I, Touitou A, Marchesani F, Marchetti M. The NMDA receptor activation by D -serine and glycine is controlled by an astrocytic Phgdh-dependent serine shuttle. 2019;
81. Shibasaki K, Hosoi N. Glycine release from astrocytes via functional reversal of GlyT1. 2017;395–403.
82. Jimenez-Blasco D, Santofimia-Castanõ P, Gonzalez A, Almeida A, Bolanõs JP. Astrocyte NMDA receptors' activity sustains neuronal survival through a Cdk5-Nrf2 pathway. Cell Death Differ. 2015;22(11):1877–89.
83. Conti F, DeBiasi S, Minelli R, Melone M. Expression of NR1 and NR2A/B subunits of the NMDA receptor in cortical astrocytes. Glia. 1996;17(3):254–8.
84. Palygin O, Lalo U, Pankratov Y. Distinct pharmacological and functional

properties of NMDA receptors in mouse cortical astrocytes. Br J Pharmacol. 2011;163(8):1755–66.

85. Luque JM, Richards JG. Expression of NMDA 2B receptor subunit mRNA in bergmann glia. Glia. 1995;13(3):228–32.
86. Alsaad HA, DeKorver NW, Mao Z, Dravid SM, Arikkath J, Monaghan DT. In the Telencephalon, GluN2C NMDA Receptor Subunit mRNA is Predominately Expressed in Glial Cells and GluN2D mRNA in Interneurons. Neurochem Res [Internet]. 2019;44(1):61–77. Available from: http://dx.doi.org/10.1007/s11064-018-2526-7
87. Araque A, Sanzgiri RP, Parpura V, Haydon PG. Calcium elevation in astrocytes causes an NMDA receptordependent increase in the frequency of miniature synaptic currents in cultured hippocampal neurons. J Neurosci. 1998;18(17):6822–9.
88. Palygin O, Lalo U, Verkhratsky A, Pankratov Y. Ionotropic NMDA and P2X1/5 receptors mediate synaptically induced Ca2+ signalling in cortical astrocytes. Cell Calcium [Internet]. 2010;48(4):225–31. Available from: http://dx.doi.org/10.1016/j.ceca.2010.09.004
89. Reimers J, Milovanovic M, Wolf M. Quantitative analysis of AMPA receptor subunit composition in addiction-related brain regions. Bone. 2008;23(1):1–7.
90. David JC, Yamada KA, Bagwe MR, Goldberg MP. AMPA receptor activation is rapidly toxic to cortical astrocytes when desensitization is blocked. J Neurosci. 1996;16(1):200–9.
91. Matthias K, Kirchhoff F, Seifert G, Hüttmann K, Matyash M, Kettenmann H, et al. Segregated expression of AMPA-type glutamate receptors and glutamate transporters defines distinct astrocyte populations in the mouse hippocampus. J Neurosci. 2003;23(5):1750–8.
92. Schröder W, Seifert G, Hüttmann K, Hinterkeuser S, Steinhäuser C. AMPA receptor-mediated modulation of inward rectifier K+ channels in astrocytes of mouse hippocampus. Mol Cell Neurosci. 2002;19(3):447–58.
93. Fiacco TA, McCarthy KD. Intracellular Astrocyte Calcium Waves In Situ Increase the Frequency of Spontaneous AMPA Receptor Currents in CA1

Pyramidal Neurons. J Neurosci. 2004;24(3):722–32.

94. Beppu K, Kosai Y, Kido MA, Akimoto N, Mori Y, Kojima Y, et al. Expression, subunit composition, and function of AMPA-type glutamate receptors are changed in activated microglia; possible contribution of GluA2 (GluR-B)-deficiency under pathological conditions. Glia. 2013;61(6):881–91.
95. Seifert G, Steinhäuser C. Glial Cells in the Mouse Hippocampus Express AMPA Receptors with an Intermediate Ca2+ Permeability. Eur J Neurosci. 1995;7(9):1872–81.
96. Kakegawa W, Katoh A, Narumi S, Miura E, Motohashi J, Takahashi A, et al. Optogenetic Control of Synaptic AMPA Receptor Endocytosis Reveals Roles of LTD in Motor Learning. Neuron [Internet]. 2018;99(5):985-998.e6. Available from: https://doi.org/10.1016/j.neuron.2018.07.034
97. Egbenya DL, Hussain S, Lai YC, Xia J, Anderson AE, Davanger S. Changes in synaptic AMPA receptor concentration and composition in chronic temporal lobe epilepsy. Mol Cell Neurosci [Internet]. 2018;92:93–103. Available from: https://doi.org/10.1016/j.mcn.2018.07.004
98. Marceau K, Ruttle PL, Shirtcliff EA, Essex MJ, Susman EJ, Studies A, et al. Structural basis of kainate subtype glutamate receptor desensitization. Nature. 2015;57(6):742–68.
99. Sommer B, Burnashev N, Verdoorn TA, Keinänen K, Sakmann B, Seeburg PH. A glutamate receptor channel with high affinity for domoate and kainate. EMBO J. 1992;11(4):1651–6.
100. Schiffer HH, Swanson GT, Heinemann SF. Rat GluR7 and a carboxy-terminal splice variant, GluR7b, are functional kainate receptor subunits with a low sensitivity to glutamate. Neuron. 1997;19(5):1141–6.
101. Jaskolski F, Coussen F, Mulle C. Subcellular localization and trafficking of kainate receptors. Trends Pharmacol Sci. 2005;26(1):20–6.
102. Reiner A, Arant RJ, Isacoff EY. Assembly Stoichiometry of the GluK2/GluK5 Kainate Receptor Complex. Cell Rep. 2012;1(3):234–40.
103. Fernandes HB, Catches JS, Petralia RS, Copits BA, Xu J, Russell TA, et al. High-Affinity Kainate Receptor Subunits Are Necessary for Ionotropic but Not

Metabotropic Signaling. Neuron [Internet]. 2009;63(6):818–29. Available from: http://dx.doi.org/10.1016/j.neuron.2009.08.010

104. Lauri SE, Vesikansa A, Segerstråle M, Collingridge GL, Isaac JTR, Taira T. Functional Maturation of CA1 Synapses Involves Activity-Dependent Loss of Tonic Kainate Receptor-Mediated Inhibition of Glutamate Release. Neuron. 2006;50(3):415–29.
105. Ben-Ari Y. Limbic seizure and brain damage produced by kainic acid: Mechanisms and relevance to human temporal lobe epilepsy. Neuroscience. 1985;14(2):375–403.
106. Beneyto M, Kristiansen L V., Oni-Orisan A, McCullumsmith RE, Meador-Woodruff JH. Abnormal glutamate receptor expression in the medial temporal lobe in schizophrenia and mood disorders. Neuropsychopharmacology. 2007;32(9):1888–902.
107. Pickard BS, Malloy MP, Christoforou A, Thomson PA, Evans KL, Morris SW, et al. Cytogenetic and genetic evidence supports a role for the kainate-type glutamate receptor gene, GRIK4, in schizophrenia and bipolar disorder. Mol Psychiatry. 2006;11(9):847–57.
108. Burnashev N, Zhou Z, Neher E, Sakmann B. Fractional calcium currents through recombinant GluR channels of the NMDA, AMPA and kainate receptor subtypes. J Physiol. 1995;485(2):403–18.
109. Huntley GW, Rogers SW, Moran T, Janssen W, Archin N, Vickers JC, et al. Selective distribution of kainate receptor subunit immunoreactivity in monkey neocortex revealed by a monoclonal antibody that recognizes glutamate receptor subunits GluR5/6/7. J Neurosci. 1993;13(7):2965–81.
110. Herb A, Burnashev N, Werner P, Sakmann B, Wisden W, Seeburg PH. The KA-2 subunit of excitatory amino acid receptors shows widespread expression in brain and forms ion channels with distantly related subunits. Neuron. 1992;8(4):775–85.
111. Brand-Schieber E, Lowery SL, Werner P. Select ionotropic glutamate AMPA/kainate receptors are expressed at the astrocyte-vessel interface. Brain Res. 2004;1007(1–2):178–82.

112. Vargas JR, Takahashi DK, Thomson KE, Wilcox KS. The expression of kainate receptor subunits in hippocampal astrocytes after experimentally induced status epilepticus. J Neuropathol Exp Neurol. 2013;72(10):919–32.
113. Sladeczek F, Pin JP, Récasens M, Bockaert J, Weiss S. Glutamate stimulates inositol phosphate formation in striatal neurones. Nature. 1985;317(6039):717–9.
114. Beqollari D, Kammermeier PJ. Venus Fly Trap Domain of mGluR1 Functions as a Dominant Negative Against Group I mGluR Signaling. 2021;439–48.
115. Sugiyama H, Ito I, Hirono C. A new type of glutamate receptor linked to inositol phospholipid metabolism. Vol. 325, Nature. 1987. p. 531–3.
116. Masu M, Tanabe Y, Tsuchida K, Shigemoto R, Nakanishi S. Sequence and expression of a metabotropic glutamate receptor. Nature. 1991;349:760–5.
117. Schools GP, Kimelberg HK. mGluR3 and mGluR5 are the predominant metabotropic glutamate receptor mRNAs expressed in hippocampal astrocytes acutely isolated from young rats. J Neurosci Res. 1999;58(4):533–43.
118. Mineff E, Valtschanoff J. Metabotropic glutamate receptors 2 and 3 expressed by astrocytes in rat ventrobasal thalamus. Neurosci Lett. 1999;270(2):95–8.
119. Zhou F, Yao H, Wu J, Yang Y-J, Ding J-H, Zhang J, et al. Activation of Group II/III Metabotropic Glutamate Receptors Attenuates LPS-Induced Astroglial Neurotoxicity Via Promoting Glutamate Uptake Fang. J Neurosci Res. 2007;84:268–77.
120. Yao HH, Ding JH, Zhou F, Wang F, Hu LF, Sun T, et al. Enhancement of glutamate uptake mediates the neuroprotection exerted by activating group II or III metabotropic glutamate receptors on astrocytes. J Neurochem. 2005;92(4):948–61.
121. Gegelashvili G, Dehnes Y, Danbolt NC, Schousboe A. The high-affinity glutamate transporters GLT1, GLAST, and EAAT4 are regulated via different signalling mechanisms. Neurochem Int. 2000;37(2–3):163–70.
122. Vermeiren C, Najimi M, Vanhoutte N, Tilleux S, De Hemptinne I, Maloteaux

JM, et al. Acute up-regulation of glutamate uptake mediated by mGluR5a in reactive astrocytes. J Neurochem. 2005;94(2):405–16.

123. He L, Vasiliou K, Nebert DW. Analysis and update of the human solute carrier (SLC) gene superfamily. Hum Genomics. 2009;3(2):195–206.
124. Levy LM, Warr O, Attwell D. Stoichiometry of the glial glutamate transporter GLT-1 expressed inducibly in a Chinese hamster ovary cell line selected for low endogenous Na+-dependent glutamate uptake. J Neurosci. 1998;18(23):9620–8.
125. Canul-Tec JC, Assal R, Cirri E, Legrand P, Brier S, Chamot-Rooke J, et al. Structure and allosteric inhibition of excitatory amino acid transporter 1. Nature. 2017;544(7651):446–51.
126. Grunewald M, Menaker D, Kanner BI. Cysteine-scanning mutagenesis reveals a conformationally sensitive reentrant pore-loop in the glutamate transporter GLT-1. J Biol Chem. 2002;277(29):26074–80.
127. Crisman TJ, Qu S, Kanner BI, Forrest LR. PNAS-2009-Crisman-Inward-facing conformation of glutamate transporters.pdf. 2009;1–6.
128. Fairman WA, Vandenberg RJ, Arriza JL, Kavanaught MP, Amara SG. An excitatory amino-acid transporter with properties of a ligand-gated chloride channel. Vol. 375, Nature. 1995. p. 599–603.
129. Arriza JL, Eliasof S, Kavanaugh MP, Amara SG. Excitatory amino acid transporter 5, a retinal glutamate transporter coupled to a chloride conductance. Proc Natl Acad Sci U S A. 1997;94(8):4155–60.
130. Arriza JL, Fairman WA, Amara SG, Kavanaugh P, Wadiche I, Murdoch H. Functional Comparisons of Three Glutamate Cloned from Human Motor Cortex Transporter. J Neurosci. 1994;14(9):5559–69.
131. Danbolt NC. Glutamate uptake. Prog Neurobiol. 2001;65(1):1–105.
132. Rothstein JD, Martin L, Levey AI, Dykes-Hoberg M, Jin L, Wu D, et al. Localization of neuronal and glial glutamate transporters. Neuron. 1994;13(3):713–25.
133. De Vries. Episodic ataxia associated with EAAT1 mutation C186S affecting glutamate reuptake (Archives of Neurology (2009) 66, 4 (497)). Arch Neurol.

2009;66(6):772.

134. Deng X, Shibata H, Takeuchi N, Rachi S, Sakai M, Ninomiya H, et al. Association study of polymorphisms in the glutamate transporter genes SLC1A1, SLC1A3, and SLC1A6 with schizophrenia. Am J Med Genet Part B Neuropsychiatr Genet. 2007;144(3):271–8.
135. Jen JC, Wan J, Palos TP, Howard BD, Baloh RW. Mutation in the glutamate transporter EAAT1 causes episodic ataxia, hemiplegia, and seizures. Neurology. 2005;65(4):529–34.
136. Shashidharan P, Huntley GW, Murray JM, Buku A, Moran T, Walsh MJ, et al. Immunohistochemical localization of the neuron-specific glutamate transporter EAAC1 (EAAT3) in rat brain and spinal cord revealed by a novel monoclonal antibody. Brain Res. 1997;773(1–2):139–48.
137. Kanai Y, Bhide P, DiFiglia M, Hediger MA. Neuronal high-affinity glutamate transport in the rat central nervous system. Neuro Rep. 1995;6:2357–62.
138. Massie A, Cnops L, Smolders I, McCullumsmith R, Kooijman R, Kwak S, et al. High-affinity Na+/K+-dependent glutamate transporter EAAT4 is expressed throughout the rat fore- and midbrain. J Comp Neurol. 2008;511(2):155–72.
139. de Vivo L, Melone M, Bucci G, Rothstein JD, Conti F. Quantitative analysis of EAAT4 promoter activity in neurons and astrocytes of mouse somatic sensory cortex. Neurosci Lett [Internet]. 2010;474(1):42–5. Available from: http://dx.doi.org/10.1016/j.neulet.2010.03.003
140. Lehre KP, Levy LM, Ottersen OP, Storm-Mathisen J, Danbolt NC. Differential expression of two glial glutamate transporters in the rat brain: Quantitative and immunocytochemical observations. J Neurosci. 1995;15(3 I):1835–53.
141. Bergles DE, Jahr CE. Synaptic activation of glutamate transporters in hippocampal astrocytes. Neuron. 1997;19(6):1297–308.
142. Chen W, Mahadomrongkul V, Berger U V, Bassan M, Desilva T, Tanaka K, et al. The Glutamate Transporter GLT1a Is Expressed in Excitatory Axon Terminals of Mature Hippocampal Neurons. 2004;24(5):1136–48.
143. Levy LM, Lehre KP, Rolstad B, Danbolt NC. A monoclonal antibody raised

against an [Na+K+]coupled l-glutamate transporter purified from rat brain confirms glial cell localization. FEBS Lett. 1993;317(1–2):79–84.

144. Mennerick S, Dhond RP, Benz A, Xu W, Rothstein JD, Danbolt NC, et al. Neuronal expression of the glutamate transporter GLT-1 in hippocampal microcultures. J Neurosci. 1998;18(12):4490–9.
145. Matsugami TR, Tanemura K, Mieda M, Nakatomi R, Yamada K, Kondo T, et al. Indispensability of the glutamate transporters GLAST and GLT1 to brain development. Proc Natl Acad Sci U S A. 2006;103(32):12161–6.
146. Schmitt A, Asan E, Püschel B, Kugler P. Cellular and regional distribution of the glutamate transporter GLAST in the CNS of rats: Nonradioactive in situ hybridization and comparative immunocytochemistry. J Neurosci. 1997;17(1):1–10.
147. Clark BA, Barbour B. Currents evoked in Bergmann glial cells by parallel fibre stimulation in rat cerebellar slices. J Physiol. 1997;502(2):335–50.
148. Danbolt NC, Storm-Mathisen J, Kanner BI. An [Na+ + K+]coupled l-glutamate transporter purified from rat brain is located in glial cell processes. Neuroscience. 1992;51(2):295–310.
149. Estrada-Sánchez AM, Rebec G V. Corticostriatal dysfunction and glutamate transporter 1 (GLT1) in Huntington's disease: Interactions between neurons and astrocytes. Basal Ganglia. 2012;2(2):57–66.
150. Swanson RA, Liu J, Miller JW, Rothstein JD, Farrell K, Stein BA, et al. Neuronal regulation of glutamate transporter subtype expression in astrocytes. J Neurosci. 1997;17(3):932–40.
151. Perego C, Vanoni C, Bossi M, Massari S, Basudev H, Longhi R, et al. The GLT-1 and GLAST glutamate transporters are expressed on morphologically distinct astrocytes and regulated by neuronal activity in primary hippocampal cocultures. J Neurochem. 2000;75(3):1076–84.
152. Lee ML, Martinez-Lozada Z, Krizman EN, Robinson MB. Brain endothelial cells induce astrocytic expression of the glutamate transporter GLT-1 by a Notch-dependent mechanism. J Neurochem. 2017;143(5):489–506.
153. Escartin C, Brouillet E, Gubellini P, Jacquard C, Smadja C, Knott GW, et al.

Ciliary Neurotrophic Factor Activates Astrocytes , Redistributes Their Glutamate Transporters GLAST and GLT-1 to Raft Microdomains , and Improves Glutamate Handling In Vivo. 2006;26(22):5978–89.

154. Estrada-Sánchez AM, Castro D, Portillo-Ortiz K, Jang K, Nedjat-Haiem M, Levine MS, et al. Complete but not partial inhibition of glutamate transporters exacerbates cortical excitability in the R6/2 mouse model of Huntington's disease. CNS Neurosci Ther. 2019;25(4):509–18.
155. Masliah E, Alford M, Mallory M, Rockenstein E, Moechars D, Van Leuven F. Abnormal glutamate transport function in mutant amyloid precursor protein transgenic mice. Exp Neurol. 2000;163(2):381–7.
156. Tanaka K, Watase K. Epilepsy and Exacerbation of Brain Injury in Mice Lacking the Glutamate Transporter GLT-1. 1997;276:1699–702.
157. Faideau M, Kim J, Cormier K, Gilmore R, Welch M, Auregan G, et al. In vivo expression of polyglutamine-expanded huntingtin by mouse striatal astrocytes impairs glutamate transport : a correlation with Huntington ' s disease subjects. 2010;19(15):3053–67.
158. Bannai S. Exchange of Cystine and Glutamate across Plasma Membrane of Human Fibroblasts *. 1986;261(5):2256–63.
159. Sato H, Tamba M, Ishii T, Bannai S. Cloning and Expression of a Plasma Membrane Cystine / Glutamate Exchange Transporter Composed of Two Distinct Proteins *. J Biol Chem. 1999;274(17):11455–8.
160. Lewerenz J, Maher P, Methner A. Regulation of xCT expression and system x À c function in neuronal cells. 2012;171–9.
161. Nakamura E, Sato M, Yang H, Miyagawa F, Harasaki M, Tomita K, et al. 4F2 (CD98) Heavy Chain Is Associated Covalently with an Amino Acid Transporter and Controls Intracellular Trafficking and Membrane Topology of 4F2 Heterodimer *. J Biol Chem. 1999;274(5):3009–16.
162. Shin C, Mishra P, Watrous JD, Carelli V, Aurelio MD, Jain M, et al. The glutamate/cystine xCT antiporter antagonizes glutamine metabolism and reduces nutrient flexibility. 2017;
163. Conrad M, Sato H. Cystine supplier and beyond The oxidative stress-

inducible cystine / glutamate antiporter , system x 2 c : cystine supplier and beyond. 2011;(March).

164. Sato H, Tamba M, Okuno S, Sato K, Keino-masu K, Masu M. Distribution of Cystine / Glutamate Exchange Transporter , System x c ⁻ , in the Mouse Brain. 2002;22(18):8028–33.
165. Re DB, Nafia I, Melon C, Shimamoto K, Goff LK, Had-aissouni L. Glutamate Leakage From a Compartmentalized Intracellular Metabolic Pool and Activation of the Lipoxygenase Pathway Mediate Oxidative Astrocyte Death by Reversed Glutamate Transport. 2006;47–57.
166. Qiu SO, Hu X, Veronique V, Bentea E, Sato H, Massie A, et al. The cystine-glutamate exchanger (xCT , Slc7a11) is expressed in significant concentrations in a subpopulation of astrocytes in the mouse brain. 2018;(December 2017):1–20.
167. Chen C, Liao S, Kuo J. Gliotoxic Action of Glutamate on Cultured Astrocytes. 2000;(160):1557–65.
168. Schubert D, Piasecki D. Oxidative Glutamate Toxicity Can Be a Component of the Excitotoxicity Cascade. 2001;21(19):7455–62.
169. Laake JH, Takumi Y, Eidet J, Torgner IA, Roberg B, Kvamme E, et al. Postembedding immunogold labelling reveals subcellular localization and pathway-specific enrichment of phosphate activated glutaminase in rat cerebellum. Neuroscience. 1999;88(4):1137–51.
170. Norenberg M, Hernandez A. FINE STRUCTURAL LOCALIZATION OF GLUTAMINE SYNTHETASE IN ASTROCYTES OF RAT BRAIN MICHAEL. 1979;161:303–10.
171. Hamberger AC, Chiang GH, Nylén ES, Scheff SW, Cotman CW. Glutamate as a CNS transmitter. I. Evaluation of glucose and glutamine as precursors for the synthesis of preferentially released glutamate. Brain Res. 1979;168(3):513–30.
172. Do KQ, Benz B, Sorg O, Pellerin L, Magistretti PJ. β-Adrenergic Stimulation Promotes Homocysteic Acid Release from Astrocyte Cultures: Evidence for a Role of Astrocytes in the Modulation of Synaptic Transmission. J

Neurochem. 1997;68(6):2386–94.

173. Bezzi P, Gundersen V, Galbete JL, Seifert G, Steinhäuser C, Pilati E, et al. Astrocytes contain a vesicular compartment that is competent for regulated exocytosis of glutamate. Nat Neurosci. 2004;7(6):613–20.
174. Li D, Agulhon C, Schmidt E, Oheim M, Ropert N. New tools for investigating astrocyte-to-neuron communication. Front Cell Neurosci. 2013;7(OCT):1–14.
175. Navarrete M, Perea G, Maglio L, Pastor J, García De Sola R, Araque A. Astrocyte calcium signal and gliotransmission in human brain tissue. Cereb Cortex. 2013;23(5):1240–6.
176. Woo DH, Han KS, Shim JW, Yoon BE, Kim E, Bae JY, et al. TREK-1 and Best1 channels mediate fast and slow glutamate release in astrocytes upon GPCR activation. Cell [Internet]. 2012;151(1):25–40. Available from: http://dx.doi.org/10.1016/j.cell.2012.09.005
177. Heller JP, Odii T, Zheng K, Rusakov DA. Imaging tripartite synapses using super-resolution microscopy. Methods [Internet]. 2020;174(May 2019):81–90. Available from: https://doi.org/10.1016/j.ymeth.2019.05.024
178. Delekate A, Fu M, Schumacher T, Ulbrich C, Foddis M, Petzold GC. astrocytic hyperactivity in vivo in an Alzheimer ' s disease mouse model. 2014;
179. Shen W, Nikolic L, Meunier C, Pfrieger F, Audinat E. An autocrine purinergic signaling controls astrocyte-induced neuronal excitation. Sci Rep [Internet]. 2017;7(1):1–13. Available from: http://dx.doi.org/10.1038/s41598-017-11793-x
180. Nagele RG, Wegiel J, Venkataraman V, Imaki H, Wang K, Wegiel J. Contribution of glial cells to the development of amyloid plaques in Alzheimer ' s disease. 2004;25:663–74.
181. Lee W, Reyes R, Gottipati M, Lewis K, Lesort M, Parpura V, et al. Enhanced Ca2+ -dependent glutamate release from astrocytes of the BACHD Huntington's disease mouse model. 2013;192–9.
182. Tian G, Azmi H, Takano T, Xu Q, Peng W, Oberheim N, et al. An astrocytic basis of epilepsy. 2007;11(9):973–81.

183. Brand-Schieber E, Werner P. AMPA/kainate receptors in mouse spinal cord cell-specific display of receptor subunits by oligodendrocytes and astrocytes and at the nodes of Ranvier. Glia. 2003;42(1):12–24.
184. Cull-Candy S, Brickley S, Farrant M. NMDA receptor subunits: Diversity, development and disease. Curr Opin Neurobiol. 2001;11(3):327–35.
185. Traynelis SF, WollmutH LP, McBain CJ, Menniti FS, Vance KM, Ogden KK, et al. Glutamate Receptor Ion Channels: Structure, Regulation, and Function Stephen. Cathet Cardiovasc Diagn. 2010;62:405–96.
186. Decrock E, De Bock M, Wang N, Gadicherla AK, Bol M, Delvaeye T, et al. IP3, a small molecule with a powerful message. Biochim Biophys Acta - Mol Cell Res [Internet]. 2013;1833(7):1772–86. Available from: http://dx.doi.org/10.1016/j.bbamcr.2012.12.016
187. Mothet JP, Pollegioni L, Ouanounou G, Martineau M, Fossier P, Baux G. Glutamate receptor activation triggers a calcium-dependent and SNARE protein-dependent release of the gliotransmitter D-serine. Proc Natl Acad Sci U S A. 2005;102(15):5606–11.
188. Takata-Tsuji F, Chounlamountri N, Do LD, Philippot C, Novion Ducassou J, Couté Y, et al. Microglia modulate gliotransmission through the regulation of VAMP2 proteins in astrocytes. Glia. 2020;(March 2019):1–12.
189. Bezzi P, Vesce S, Rossi D, Rizzini BL, Volterra A, Carmignoto G, et al. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. Nature [Internet]. 1998;391(6664):281–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9440691
190. Zhang Q, Fukuda M, Van Bockstaele E, Pascual O, Haydon PG. Synaptotagmin IV regulates glial glutamate release. Proc Natl Acad Sci U S A. 2004;101(25):9441–6.
191. Reiner A, Levitz J. Glutamatergic Signaling in the Central Nervous System: Ionotropic and Metabotropic Receptors in Concert. Neuron [Internet]. 2018;98(6):1080–98. Available from: https://doi.org/10.1016/j.neuron.2018.05.018
192. Marcaggi P, Mutoh H, Dimitrov D, Beato M, Knöpfel T. Optical measurement

of mGluR1 conformational changes reveals fast activation, slow deactivation, and sensitization. Proc Natl Acad Sci U S A. 2009;106(27):11388–93.

193. Batchelor A, Garthwaite J. Frequency detection and temporally dispersed synaptic signal association through a metabotropic glutamate receptor pathway. Nature. 1997;385:74–7.
194. Han J, Kesner P, Metna-Laurent M, Duan T, Xu L, Georges F, et al. Acute cannabinoids impair working memory through astroglial CB 1 receptor modulation of hippocampal LTD. Cell [Internet]. 2012;148(5):1039–50. Available from: http://dx.doi.org/10.1016/j.cell.2012.01.037
195. Min R, Nevian T. Astrocyte signaling controls spike timing-dependent depression at neocortical synapses. Nat Neurosci. 2012;15(5):746–53.
196. Martín R, Bajo-Grañeras R, Moratalla R, Perea G, Araque A. Circuit-specific signaling in astrocyte-neuron networks in basal ganglia pathways. Science (80-). 2015;349(6249):730–4.
197. Perea G, Gómez R, Mederos S, Covelo A, Ballesteros JJ, Schlosser L, et al. Activity-dependent switch of gabaergic inhibition into glutamatergic excitation in astrocyte-neuron networks. Elife. 2016;5(DECEMBER2016):1–26.
198. Kang J, Jiang L, Goldman SA, Nedergaard M. Astrocyte-mediated potentiation of inhibitory synaptic transmission. Nat Neurosci. 1998;1(8):683–92.
199. Rudolph R, Jahn HM, Courjaret R, Messemer N, Kirchhoff F, Deitmer JW. The inhibitory input to mouse cerebellar Purkinje cells is reciprocally modulated by Bergmann glial P2Y1 and AMPA receptor signaling. Glia. 2016;64(7):1265–80.
200. Gómez-Gonzalo M, Martin-Fernandez M, Martínez-Murillo R, Mederos S, Hernández-Vivanco A, Jamison S, et al. Neuron–astrocyte signaling is preserved in the aging brain. Glia. 2017;65(4):569–80.
201. Gómez-Gonzalo M, Zehnder T, Requie LM, Bezzi P, Carmignoto G. Insights into the release mechanism of astrocytic glutamate evoking in neurons NMDA receptor-mediated slow depolarizing inward currents. Glia. 2018;66(10):2188–99.

202. Corkrum M, Rothwell PE, Thomas MJ, Kofuji P, Araque A. Opioid-Mediated Astrocyte–Neuron Signaling in the Nucleus Accumbens. Cells. 2019;8(6):586.
203. Araque A, Parpura V, Sanzgiri RP, Haydon PG. Glutamate-dependent astrocyte modulation of synaptic transmission between cultured hippocampal neurons. Eur J Neurosci. 1998;10(6):2129–42.
204. Pirttimaki TM, Hall SD, Parri HR. Sustained neuronal activity generated by glial plasticity. J Neurosci. 2011;31(21):7637–47.
205. Iglesias R, Dahl G, Qiu F, Spray DC, Scemes E. Pannexin 1: The molecular substrate of astrocyte “hemichannels.” J Neurosci. 2009;29(21):7092–7.
206. Sáez JC, Vargas AA, Hernández DE, Ortiz FC, Giaume C, Orellana JA. Permeation of molecules through astroglial connexin 43 hemichannels is modulated by cytokines with parameters depending on the permeant species. Int J Mol Sci. 2020;21(11):1–22.
207. Sánchez OF, Rodríguez A V., Velasco-España JM, Murillo LC, Sutachan J-J, Albarracin S-L. Role of Connexins 30, 36, and 43 in Brain Tumors, Neurodegenerative Diseases, and Neuroprotection. Cells. 2020;9(4):846.
208. Nagy JI, Li W, Hertzberg EL, Marotta CA. Elevated connexin43 immunoreactivity at sites of amyloid plaques in Alzheimer’s disease. Brain Res. 1996;717(1–2):173–8.
209. Mei X, Ezan P, Giaume C, Koulakoff A. Astroglial connexin immunoreactivity is specifically altered at β-amyloid plaques in β-amyloid precursor protein/presenilin1 mice. Neuroscience [Internet]. 2010;171(1):92–105. Available from: http://dx.doi.org/10.1016/j.neuroscience.2010.08.001
210. Kajiwara Y, Wang E, Wang M, Sin WC, Brennand KJ, Schadt E, et al. GJA1 (connexin43) is a key regulator of Alzheimer’s disease pathogenesis. Acta Neuropathol Commun. 2018;6(1):144.
211. Wang Y, Wu Z, Liu X, Fu Q. LaminaGastrodin ameliorates Parkinson’s disease by downregulating connexin 43. Mol Med Rep. 2013;8(2):585–90.
212. Fujita A, Yamaguchi H, Yamasaki R, Cui Y, Matsuoka Y, Yamada K ichi, et al. Connexin 30 deficiency attenuates A2 astrocyte responses and induces

severe neurodegeneration in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride Parkinson's disease animal model. J Neuroinflammation. 2018;15(1):1–20.

213. Almad AA, Doreswamy A, Gross SK, Richard JP, Huo Y, Haughey N, et al. Connexin 43 in astrocytes contributes to motor neuron toxicity in amyotrophic lateral sclerosis. Glia. 2016;64(7):1154–69.
214. Spitale FM, Vicario N, Rosa M Di, Tibullo D, Vecchio M, Gulino R, et al. Increased expression of connexin 43 in a mouse model of spinal motoneuronal loss. Aging (Albany NY). 2020;12(13):12598–608.
215. Kirichenko EY, Salah M. M S, Goncharova ZA, Nikitin AG, Filippova SY, Todorov SS, et al. Ultrastructural evidence for presence of gap junctions in rare case of pleomorphic xanthoastrocytoma. Ultrastruct Pathol [Internet]. 2020;44(2):227–36. Available from: https://doi.org/10.1080/01913123.2020.1737609
216. Bates DC, Sin WC, Aftab Q, Naus CC. Connexin43 Enhances Glioma Invasion by a Mechanism Involving the Carboxy Terminus. 2007;1564(April):1554–64.
217. Metrics GH. Global , regional , and national incidence , prevalence , and years lived with disability for 354 diseases and injuries for 195 countries and territories , 1990 – 2017 : a systematic analysis for the Global Burden of Disease Study 2017. 2019;1990–2017.
218. Lavretsky H. HISTORY OF SCHIZOPHRENIA AS A PSYCHIATRIC DISORDER. 2008;(1863):3–13.
219. Patel KR, Cherian J, Gohil K. Schizophrenia : Overview and Treatment Options. 2014;39(9):638–45.
220. Laursen TM, Nordentoft M, Mortensen PB. Excess Early Mortality in Schizophrenia. 2014;
221. Shenton ME, Dickey CC, Frumin M, Mccarley RW. A review of MRI findings in schizophrenia. 2010;49:1–52.
222. Mccarley RW, Wible CG, Frumin M, Hirayasu Y, Levitt JJ, Fischer IA, et al. MRI Anatomy of Schizophrenia. 2010;45(9):1099–119.

223. Flores G, Morales-medina JC, Diaz A. Neuronal and brain morphological changes in animal models of schizophrenia. Behav Brain Res. 2016;301:190–203.
224. Harrison PJ. The neuropathology of schizophrenia A critical review of the data and their interpretation. 1999;593–624.
225. Siever LJ, Koenigsberg HW, Harvey P, Mitropoulou V, Laruelle M, Abi-dargham A, et al. Cognitive and brain function in schizotypal personality disorder. 2002;54:157–67.
226. Harrison PJ. Schizophrenia genes , gene expression , and neuropathology : on the matter of their convergence. 2005;40–68.
227. Mcdonald C, Murray RM. Early and late environmental risk factors for schizophrenia 1. 2000;(October 1999):130–7.
228. Polderman TJC, Benyamin B, Leeuw CA De, Sullivan PF, Bochoven A Van, Visscher PM, et al. A n a ly s i s Meta-analysis of the heritability of human traits based on fifty years of twin studies. Nat Publ Gr. 2015;47(7):702–9.
229. Faa G, Manchia M, Pintus R, Gerosa C, Marcialis MA, Fanos V. Review Fetal Programming of Neuropsychiatric Disorders. 2016;
230. Pasamanick B, Rogers M. PREGNANCY EXPERIENCE AND DISORDER THE DEVELOPMENT OF BEHAVIOR DISORDER IN CHILDREN. 1956;
231. Sinha S, Sims D, Davies J, Chiswick M. RELATION BETWEEN PERIVENTRICULAR HEMORRHAGE AND ISCHEMIC BRAIN LESIONS DIAGNOSED BY ULTRASOUND IN VERY PRE-TERM INFANTS. Lancet. 1985;1154–6.
232. Jacobsen B, Kinney K. Perinatal complications in adopted and non-adopted schizophrenics and their controls : Preliminary results. 1980;
233. Castro-rodríguez DC, Ibáñez CA, Uribe J, Menjivar M, Ángeles MDL, Gerow GKG, et al. Strengths and validity of three methods for assessing rat body fat across the life course. Int J Obes. 2020;
234. Al-gubory H. Environmental pollutants and lifestyle factors induce oxidative stress and poor prenatal development. 2014;17–31.
235. Lyall K, Schmidt RJ, Hertz-picciotto I. Original article Maternal lifestyle and

environmental risk factors for autism spectrum disorders. 2014;(February):443–64.

236. Hunter DS, Hazel SJ, Kind KL, Owens JA, Pitcher JB, Gatford KL. Physiology & Behavior Programming the brain : Common outcomes and gaps in knowledge from animal studies of IUGR. Physiol Behav. 2016;164:233–48.
237. Pugliese V, Bruni A, Anna E, Calabrò G, Cerminara G, Sampogna G, et al. Maternal stress , prenatal medical illnesses and obstetric complications : Risk factors for schizophrenia spectrum disorder , bipolar disorder and major depressive disorder. Psychiatry Res. 2019;271(November 2018):23–30.
238. Gejman P, Sanders A, Duan J. The role of genetics in the etiology of schizophrenia. 2010;33(1):1–30.
239. Gregório SP, Sallet PC, Do K, Lin E, Gattaz WF, Dias-neto E. Polymorphisms in genes involved in neurodevelopment may be associated with altered brain morphology in schizophrenia : Preliminary evidence. Psychiatry Res. 2009;165(1–2):1–9.
240. Åberg K, Saetre P, Jareborg N, Jazin E. Human QKI , a potential regulator of mRNA expression of human oligodendrocyte-related genes involved in schizophrenia. 2006;
241. Kahler A, Djurovic S, Kulle B, Jo EG, Agartz I, Ka AK, et al. Association Analysis of Schizophrenia on 18 Genes Involved in Neuronal Migration : MDGA1 as a New Susceptibility Gene. 2008;1100:1089–100.
242. Corfas G, Roy K, Buxbaum JD. Neuregulin 1-erbB signaling and the molecular / cellular basis of schizophrenia. 2004;7(6):575–80.
243. Ozaki M, Sasner M, Yano R, Lu HS, Buonanno A. letters to nature Neuregulin- NL induces expression of an NMDA-receptor subunit. 1997;691–4.
244. Rieff HI, Raetzman LT, Sapp DW, Yeh HH, Siegel RE, Corfas G. Neuregulin Induces GABA A Receptor Subunit Expression and Neurite Outgrowth in Cerebellar Granule Cells. 1999;19(24):10757–66.
245. Liu Y, Ford B, Mann MA, Fischbach GD. Neuregulins Increase ⎵ 7 Nicotinic

Acetylcholine Receptors and Enhance Excitatory Synaptic Transmission in GABAergic Interneurons of the Hippocampus. 2001;21(15):5660–9.

246. Canoll PD, Musacchio JM, Hardy R, Reynolds R, Marchionni MA, Salzer JL. GGF / Neuregulin Is a Neuronal Signal That Promotes the Proliferation and Survival and Inhibits the Differentiation of Oligodendrocyte Progenitors. 1996;17:229–43.
247. Olney JW, Newcomer JW, Farber NB. NMDA receptor hypofunction model of schizophrenia. 1999;33:523–33.
248. Javitt C. Recent Advances in the phencyclidine model of Schizophrenia. 1991;(October):1301–8.
249. Lisek M, Ferenc B, Studzian M, Pulaski L, Guo F. Glutamate Deregulation in Ketamine-Induced Psychosis — A Potential Role of PSD95 , NMDA Receptor and PMCA Interaction. 2017;11(June):1–17.
250. Moghaddam B, Adams B, Verma A, Daly D. Activation of Glutamatergic Neurotransmission by Ketamine : A Novel Step in the Pathway from NMDA Receptor Blockade to Dopaminergic and Cognitive Disruptions Associated with the Prefrontal Cortex. 1997;17(8):2921–7.
251. Pham TH, Defaix C, Mai T, Nguyen L, Mendez-david I, Tritschler L, et al. Pharmacology , Biochemistry and Behavior Cortical and raphe GABA A , AMPA receptors and glial GLT-1 glutamate transporter contribute to the sustained antidepressant activity of ketamine. Pharmacol Biochem Behav. 2020;192(December 2019):172913.
252. Adams B, Moghaddam B, Haven W. Dissociated from the Cognitive and Locomotor Effects of Phencyclidine. 1998;18(14):5545–54.
253. Eastwood SL, Harrison PJ. Decreased expression of vesicular glutamate transporter 1 and complexin II mRNAs in schizophrenia : further evidence for a synaptic pathology affecting glutamate neurons. 2005;73:159–72.
254. Zink M, Vollmayr B, Gebicke-haerter PJ, Henn FA. Neuropharmacology Reduced expression of glutamate transporters vGluT1 , EAAT2 and EAAT4 in learned helpless rats , an animal model of depression. Neuropharmacology. 2010;58(2):465–73.

255. Shan D, Lucas EK, Drummond JB, Haroutunian V, Meador-woodruff JH, Mccullumsmith RE. Abnormal expression of glutamate transporters in temporal lobe areas in elderly patients with schizophrenia. Schizophr Res. 2013;144(1–3):1–8.
256. Mccullumsmith RE, Donovan SMO, Drummond JB, Benesh FS, Simmons M, Roberts R, et al. Cell-speci fi c abnormalities of glutamate transporters in schizophrenia : sick astrocytes and compensating relay neurons ? 2016;(March 2015):823–30.
257. Bauer D, Haroutunian V, Meador-woodruff JH, Mccullumsmith RE. Abnormal glycosylation of EAAT1 and EAAT2 in prefrontal cortex of elderly patients with schizophrenia. Schizophr Res. 2010;117(1):92–8.
258. Bauer D, Gupta D, Harotunian V, Meador-woodruff JH, Mccullumsmith RE. Abnormal expression of glutamate transporter and transporter interacting molecules in prefrontal cortex in elderly patients with schizophrenia ☆. 2008;104:108–20.
259. Haberny KA, Paule MG, Scallet AC, Sistare FD, Lester DS, Hanig JP, et al. Ontogeny of the N-Methyl-D-Aspartate (NMDA) Receptor System and Susceptibility to Neurotoxicity. 2002;17:9–17.
260. Roo M De, Klauser P, Briner A, Nikonenko I, Mendez P, Kiss JZ, et al. Anesthetics Rapidly Promote Synaptogenesis during a Critical Period of Brain Development. 2009;4(9):1–9.
261. Koh MT, Shao Y, Rosenzweig-Lipson S, Gallagher M. Treatment with levetiracetam improves cognition in a ketamine rat model of schizophrenia. Schizophr Res. 2018;193:119–25.
262. Frohlich J, Van J. NIH Public Access. 2014;28(4):287–302.
263. Hauser MJ, Isbrandt D, Roeper J. Disturbances of novel object exploration and recognition in a chronic ketamine mouse model of schizophrenia. Behav Brain Res. 2017;332:316–26.
264. Zhu X, Ye G, Wang Z, Luo J, Hao X. Neuroscience Letters Sub-anesthetic doses of ketamine exert antidepressant-like effects and upregulate the expression of glutamate transporters in the hippocampus of rats. Neurosci

Lett. 2017;639:132–7.

265. Zhu X, Ye G, Wang Z, Luo J, Hao X. Sub-anesthetic doses of ketamine exert antidepressant-like effects and upregulate the expression of glutamate transporters in the hippocampus of rats. Neurosci Lett. 2016;

Annexed

***Manuscript submitted for review for publication.**

"Revealing the contribution of astrocytes to glutamatergic information processing".

Keywords: Gliotransmission, Glutamate, Connexins, Calcium, P2X, ATP, Purinergic signaling.

Abstract

Traditionally, glutamatergic neurotransmission primarily focused on the role of presynaptic and postsynaptic neurons, while astrocytes were thought only to provide what neurons need to ensure successful information flow. However, new evidence indicates that astrocytes contribute actively and even can regulate neuronal transmission at different levels. This review first provides a detailed description of the different glutamatergic components in both neurons and astrocytes. Next, we describe the evidence indicating that astrocytes might modulate or otherwise influence neuronal transmission in a more active and sophisticated way than previously thought. Finally, we elaborate on how extracellular ATP, together with glutamate, emerges as a key component that allows astrocyte modulation of energy metabolism, synaptic transmission, and cellular response during physiological and pathological conditions. The discoveries about astrocytes' role in glutamatergic neurotransmission have allowed us to better understand the complex network of neuron-astrocyte interactions. However, the contribution of astrocytes during specific behaviors remains to be elucidated. A full understanding of the astrocyte's role in physiological and pathophysiological processes such as neurodegenerative diseases will greatly impact the development of new therapeutic applications.