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Glial reactivity after antipsychotic treatment. An experimental study in rats and its implications for psychiatry

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Introduction. The importance of the glial cells in the function of the nervous system and in its pathology has been the object of multiple studies in the last years. Specifically, their role in the action of the antipsychotics is debated. Our study has analyzed glial reactivity in rats treated with antipsychotics.

Methodology. In a first ultrastructural study of the arcuate nucleus of the hypothalamus, the animals were treated with chlorpromazine for 40 days, and were sacrificed at the end of the treatment, after 20 days of rest without treatment. In another series of studies, with the light microscope and immunohistochemistry we evaluated the immunoreactivity of the glial fibrillary acidic protein (GFAP) in six regions of the central nervous system of rats treated with typical and atypical antipsychotics.

Results. With the electron microscope, the animals treated with chlorpromazine showed a significant reduction of the axosomatic synapses on the neurons of the hypothalamic arcuate nucleus and an increase of glial presence, as noted by the greater amount of astrocyte processes. The mentioned modifications were reversible, tending to normalize in a group of animals sacrificed 20 days after completion of the treatment. In the immunohistochemical study, the glial reaction was important in the territory of the nucleus accumbens with all the antipsychotics, moderate in the cingulate cortex, although only with atypical antipsychotics, and scarcely significant in the rest of the regions.

Conclusions. Our results confirm that the glial cells are targets of the antipsychotic action, and this will allow us to better understand the action of these drugs and the role of the glial cells in the normal function of the nervous system and in the mental disease.

Key words:
Antipsychotics, astrocytes, GFAP

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Reactividad glial tras el tratamiemto con antipsicóticos. Estudio experimental en ratas e implicaciones para la psiquiatría

Introducción. La importancia de las células gliales en la función del sistema nervioso y en su patología ha sido objeto de múltiples estudios en los últimos años. Concretamente se debate su papel en la acción de los antipsicóticos. Nuestro estudio analiza la reactividad glial en ratas tratadas con antipsicóticos.

Metodología. En un primer estudio ultraestructural del núcleo arcuato del hipotálamo, los animales fueron tratados con clorpromacina durante 40 días, sacrificándose al final del tratamiento y tras 20 días de descanso. En otra serie de estudios, con el microscopio de luz y con técnicas inmunohistoquímicas valoramos la reacción a la proteína glial fibrilar ácida (GFAP) en seis regiones del sistema nervioso central de ratas tratadas con antipsicóticos típicos y atípicos.

Resultados. Con el microscopio electrónico, las ratas tratadas mostraron una reducción significativa de las sinapsis axosomáticas sobre las neuronas del núcleo arcuato del hipotálamo, así como un incremento de la presencia glial evidenciable por la mayor cantidad de laminillas de astrocitos. Las modificaciones mencionadas son reversibles, tendiendo a normalizarse en los animales sacrificados a los 20 días de finalizado el tratamiento. En el estudio inmunohistoquímico la reacción astrocitaria fue muy importante en el territorio del núcleo accumbens con todos los antipsicóticos, moderada en la corteza cingular, aunque sólo con los atípicos, y discreta en el resto de las regiones.

Conclusiones. Nuestros resultados confirman que las células gliales son diana de los antipsicóticos, lo que ha de contribuir a entender mejor la acción de estos fármacos y el papel de las células gliales en el normal funcionamiento del sistema nervioso y en la enfermedad mental.

Palabras claves:
Antipsicóticos, astrocitos, GFAP.

INTRODUCTION

In the middle of the 19th century, Virchow described the glia for the first time, assigning it a role of "nerve glue." Since then, and for more than one century, much information has become known on these cells, although the view that assigned them a secondary role, to the service of the neurons, always prevailed. In the central nervous system (CNS), the astroglia, oligodendroglia and microglia are distinguished. The first one of these, the astrocytes, is the most polyvalent and has the closest relationship with the neurons.

Now focusing on the astrocytes, we bring to mind that until recently their participation was considered to exist in functions having some importance (support, isolation or nutrition of the neurons, and their guide when they migrate to their destinations during development; uptake and elimination of the transmitters released in the synapses; regulation of the extracellular levels of ions, participation in the formation of the blood-brain barrier, etc.), although accessory, because they excluded the principal function of information processing.

But this is also changing. In recent years, different forms of signal exchange have been identified in both directions between the neurons and glial cells[1-4]. We currently know that the macroglial cells, and especially the astrocytes, express ion channels and G protein linked receptors as well as receptors for many of the neurotransmitters. This makes it possible for them to receive information from the neighboring neurons. In response to that information, the glial cells modify their calcium levels since they relay their own messages into the extracellular space (glutamate, prostaglandins, nitric acid, ATP or growth factors), which reach other glial cells as well as the neighboring neurons, modulating their activity (gliotransmission)[5-7].

The evidence that the glia plays an active role in the formation, maturation, maintenance and level of the activity of the synapses is of special importance[8-11], up to the point that without the glia, the neurons would not carry out their functions. And, if the above were not sufficient, we also know that the so-called neural stem cells are of glial strain (or at least they share certain markers with the glia), maintaining the capacity to generate new glial cells and neurons in some territories of the CNS such as the subventricular zone or the hippocampus[12]. The new paradigm manifests that the functional units of the nervous system is not simply the neuron but that is also formed by the association of the neuron and glia.

As a consequence of these advances, that manifests the importance of the glia in the nervous system functions, it also becomes clear that the alterations of the glial cells are found in the origin of or are related to many nervous system diseases. Furthermore, we propose that the glia could be the target of the drugs used in the treatment of neurological and mental diseases, both those that are used as those that could be designed in the future.

With these backgrounds, we propose the study of the repercussions that antipsychotic treatment may have on the glial-neuronal relationship, on the synaptic connections and the glial cells. This study aims to improve our knowledge of the action of antipsychotics and the role of the glia in the mental conditions.

METHODOLOGY

All the animals used in the present study were treated according to the European Community guidelines and those of the government of Spain regarding the care of experimental animals. We used Wistar albino rats. All the animals were subjected to a 12/12 hour light/dark cycle from birth, in a room with steady state temperatures (18-20ºC) and controlled humidity and had food and water ad libitum.

Both the treated as well as control group of animals included 5 subjects, except when indicated otherwise. In order to study the arcuate nucleus of the hypothalamus of the rat treated with chlorpromazine for 40 days with the electron microscope, the animals, adult males, were divided into the following groups: treated rats (daily intramuscular injection), control rats (intramuscular injection with distilled water) and rats that were treated and sacrificed at 20 days of the end of the treatment (treated plus rest), in order to analyze the reversibility of the possible changes. The chlorpromazine dose administered was established progressively, going from 8 to 14 mg/K weight/day between days one and thirteen of treatment, which corresponds to a high range of the dose used in humans. After anesthesia, the animals were perfused via vascular with glutaraldehyde at 2% and paraformaldehyde at 2% in phosphate buffer 0.1M, pH 7.4. Then, the hypothalamuses of all the animals were dehydrated embedded in araldite. The ultrafine cuts were studied and microphotographs were obtained with an electron microscope Zeiss EM 900.

We quantified the number of axosomatic synapses on the arcuate neurons in these animals. To do so, for each one of the animals selected, a grid field was randomly chosen and we performed a counting in the microscope screen, with 30,000x magnification. We decided to study the axosomatic synapses, so that the values are expressed as synapsis number/neuronal somata. To do so, we especially considered the fact that these neuronal somata are a homogeneous and comparable population. Thus, only those somata having a similar size in which the cell nucleus was well-represented in the section were counted. For the processing of the data obtained, the Analysis of the Variance (ANOVA) was used.

In another series of experiments, the animals were treated for 30 days with clomipramine and with typical antipsychotics (chlorpromazine, haloperidol) and atypical ones (risperidone, olanzapine, ziprasidone). All the brains of the animals, both treated and controls, were embedded in paraffin according to the conventional techniques of optic microscopy. The coronal sections of the CNS were stained using an immunohistochemical method to show the presence of the glial fibrillary acidic protein (GFAP) in the following territories: striatum, hypothalamus, hippocampus, cingulate cortex, amygdala and nucleus accumbens. The primary antiserum was obtained from Dako. We used the kit *EnVision doublestain system* of Dako as secondary regents. As control test, the primary antiserum was omitted or substituted by normal serum. The stained sections were studied and photographed in a Nikon Eclipse90i microscope.

RESULTS

Ultrastructure study of the rats treated with chlorpromazine

The most outstanding findings with the electron microscope were, in our opinion, the important presence of glial cells and especially the development of the processes or lamellae of the astrocytes that ensheath the neuronal somata (fig. 1A), sometimes forming several layers that seem to isolate and disconnect the neurons (fig. 1B). It is also common to observe dendrites completely ensheathed by glial lamellae.

The axosomatic synapses were counted with 100 arcuate neurons per group of rats and therefore expressed as the number of synaptic contacts per neuronal somata. Figure 2 summarizes the data of the different groups of animals identified as C (controls), T (treated), C+R (controls that rested 20 days before being sacrificed), and T+R (treated with chlorpromazine and sacrificed at 20 days of completing the treatment. The reduction in the number of synapsis following the antipsychotic treatment (from 5.86 to 2.35 on an average per neuron) and a partial recovery in the rats who rested before being sacrificed (up to 4.1) stands out.

Immunohistochemical study of glial reactivated

As we have indicated in the section on material and techniques, we have analyzed the reactivity to GFAP in six large regions of the CNS (hypothalamus, amygdala, cingulate cortex, hippocampus, striatum and accumbens) in control rats and rats treated with two typical antipsychotics (chlorpromazine and haloperidol) and three atypical ones (risperidone, olanzapine and ziprasidone). We obtained a large number of images, so that not all of them can be shown. Therefore, we have only chosen some representative examples.

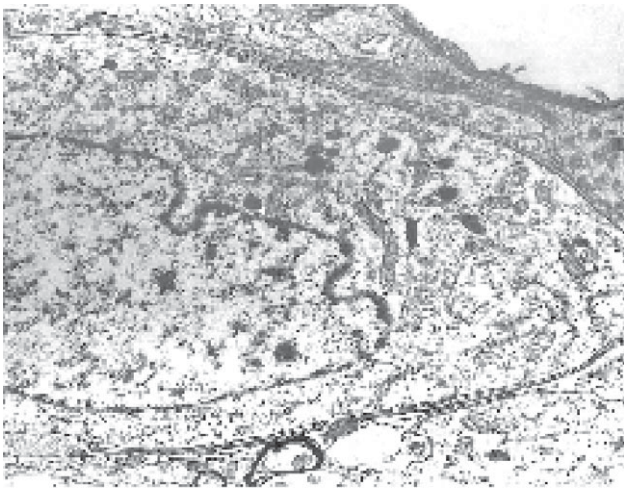


Figure 1A: 7.000 X

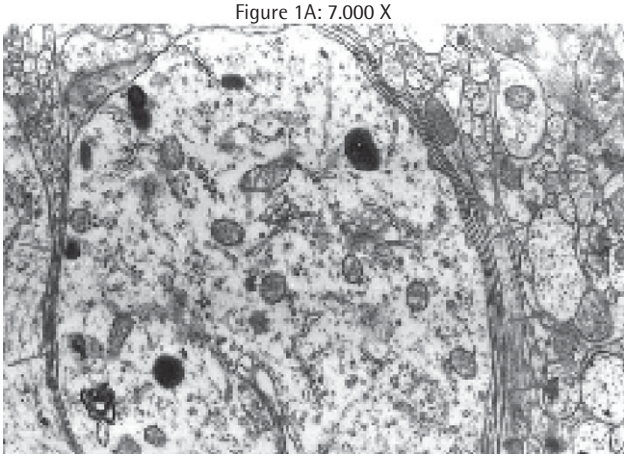
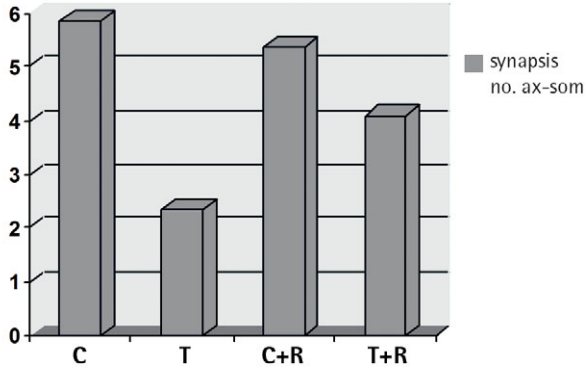


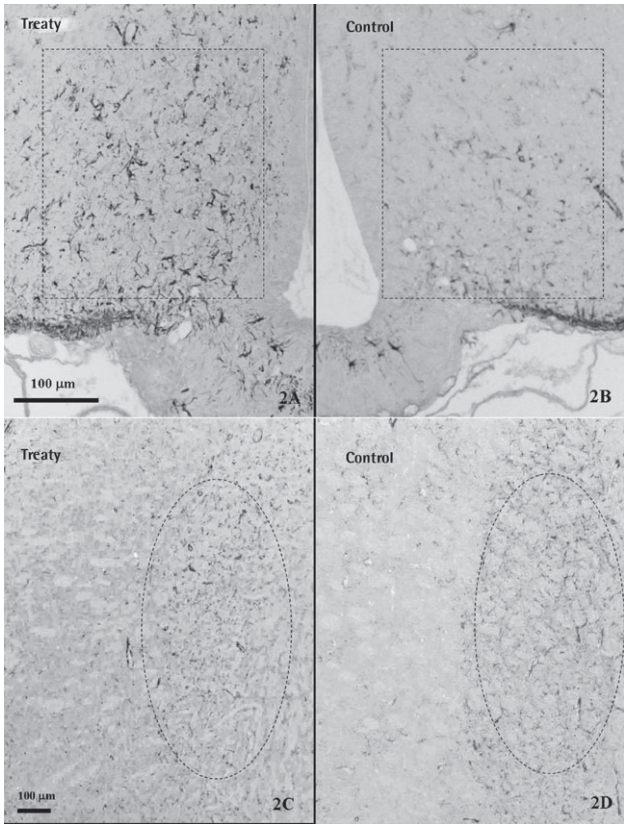
Figure 1B: 12.000 X
The neurons are surrounded by glial laminae that isolate them.

Figura 1 — Ultrastructure of the arcuate neurons of rats treated with chlorpromazine



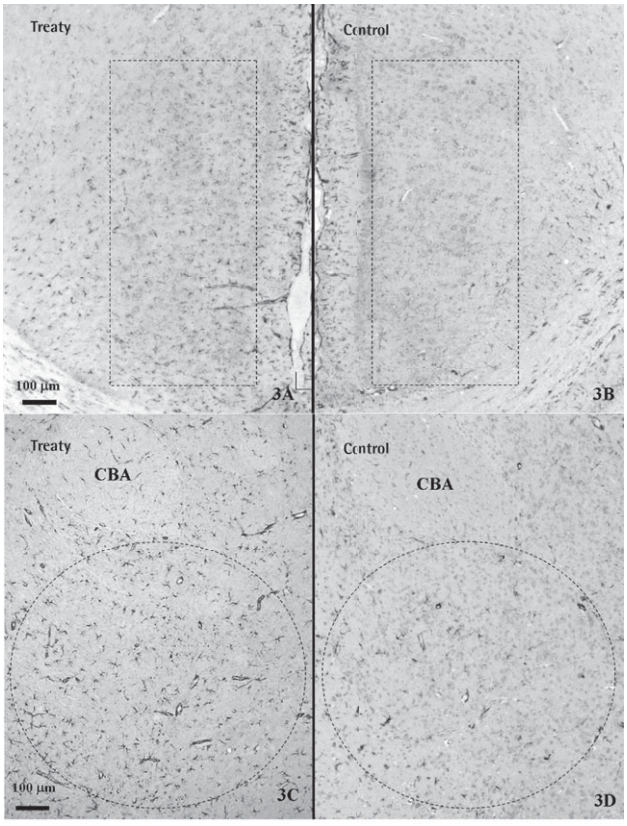
C = controls; T = treated; C+R = controls and sacrificed after rest; T+R = Treated and sacrificed after rest.

Figure 2 — Quantification of the axosomatic synapses on the neurons of the arcuate nucleus of rats treated with chlorpromazine



2A and B correspond to hypothalamus; 2C and D correspond to striatum. Similar territories have been marked for their comparison.

Figure 3 Immunoreactivity to GFAP in rats treated with risperidone



The sections of 3C and D are of the territory of the nucleus accumbens. Similar territories have been marked for their comparison.

Figure 4 Immunoreactivity to GFAP in rats treated with risperidone

Perhaps the best way to express the results would be the comparative summary offered in table 1. This evaluates reactivity to the glial protein in relation to the controls, according to our experience and as an average of the entire iconography made. As can be seen, all of the antipsychotics lead to a significant increase in the labeling on the nucleus accumbens and a moderate one on the hypothalamus and cingulate cortex. The glial reaction is more discrete in other territories such as the amygdala and hippocampus, and the striatum, where it seems to be greater after treatment with haloperidol.

Figures 3 and 4 show our results after the administration of risperidone (table 1 shows that very similar results were obtained after treatment with olanzapine or ziprasidone). In these figures, the image corresponding to the treated animal is located to the left of that obtained for the control rats. To simplify the comparison, we have outlined similar territories to the same increases in both groups. Figures 3 (2A y B) compare the reaction to GFAP in the mediobasal hypothalamus, specifically in the arcuate nucleus. The astrocyte processes, especially those surrounding the vessels, are more evident in the treated rats. The reactive glia in the striatum is shown in figures 3 (2C y D), it being possible to verify that there are no differences in this region between the control rats and those treated with risperidone.

In figure 4, we summarize the findings in the cingulate cortex (3A, B) and in the nucleus (3C, D). In the control animals, the GFAP-labeled cells are scarce in the dorsal anterior cingulate cortex of the callous body (fig. 4, 3B), where a moderate reaction appears in the most superficial layers, but is very limited in the deeper layers. The same can be said about the region of the nucleus accumbens ventral to the anterior white commissure (AWC) (fig. 4, 3D). On the contrary, in the rats treated with Risperidone for 30 days, in images similar to those shown for the control rats, the glial cells are much more numerous in the same territories (fig. 4, 3A and 3C), although, in the accumbens, the labeling predominates in the zone ventral to the AWC, and in the

Table 1 Summary of the reactivity to GFAP after the different treatments

	Striatum	Accumbens	Hippocampus	Amygdala	Cingulate cortex	Hypothalamus
Chlorpromazine	-	+ + +	-	-	-	+
Haloperidol	+ +	+ + +	-	-	-	+
Risperidone	+	+ + +	+	+	+ +	+ +
Olanzapine	-	+ + +	+	+	+ +	+
Ziprasidone	+	+ + +	+	+	+ +	+ +

- Without noticeable differences with the control; + Discreet increased reactivity; ++ Moderately increased reactivity; +++ Very increased reactivity

frontal cortex, the increase in the number of astrocytes is limited to the deep layers of the cortex, no differences in the most superficial layer being observed.

DISCUSSION

As the glia acquires the importance and recognition it should have because of its important functional role, it has been becoming clear that these cells occupy an important place within neurological and mental conditions (their involvement may be a determining factor for the development of the disease) and in the therapy used to treat these disorders.

It has been known for some time that antipsychotic treatment induces an increase in glial presence[13, 14], although initially no importance was given to this finding within the dominant paradigm. However, with the current knowledge, that shows that glial cells regulate the microenvironment and almost all of the neuronal functions, knowing that the glial cells have receptors for many neurotransmitters, which converts them into a target of the psychopharmaceuticals, old problems must be reviewed and new proposals made.

In the literature, many works are found that describe a glial deficit in schizophrenia[15-18]. In order to illustrate the importance of the role that may be played by glial cells, specifically the astrocytes, in this disease, we are going to review some recent proposals on its possible role[19]. The hypotheses that have attempted to explain the pathophysiology of this condition originate in pharmacology. Thus, the hyperdopaminergic hypothesis comes from, above all, the identification of the D2 receptor blockade as an action mechanism of the first antipsychotics, and is supported by the contrasted fact that the stimulants that act via dopamine (amphetamines) may produce signs of psychosis in normal individuals as well as exacerbate the symptoms of schizophrenia. The evidence that postulates a role for glutamate in this disease (hypoglutamatergic hypothesis) also comes from pharmacology, since certain antagonists of the NMDA receptors (phencyclidine) produce signs of psychosis that are similar to schizophrenia[20].

It is currently well established that glutamate is the most frequent excitation-producing neurotransmitter in the CNS and it is not casual that practically all the glutamate-linked functions are controlled by the glia. Maintenance of glutamate levels is a task that is carried out by the astrocyte and is a key function for the normal functioning of the CNS. Glutamate, once released, must be rapidly removed from the extracellular space since, on the contrary, it will be toxic for the neurons per se, so that it is captured by the astrocytes (whose membranes contain most of the glutamate transporters) where it is transformed into glutamine, which is sent back to the neurons to form new glutamate (it is the glutamate/glutamine cycle)[21]. In this way, an excess of glutamate transporter action in the cortex of schizophrenics would be translated into hypofunction of the neurotransmitter in the cortex[22].

On the other hand, the aminergic systems of the brain stem, as dopaminergic, are under control of the cortical glutamatergic neurons, so that if the glutamate action decreases in the cortex, striatal dopamine release is increased. Seemingly, these nervous circuits interact by negative feedback, with which the striatal hyperdopaminergia weakens the glutamate function in cortex more[23, 24]. In conclusion, an abnormality in the glial cells of the cortex is translated into a cascade of alterations that may involve severe neurotransmitter systems and circuits in other regions of the CNS.

We must point out that our initial proposal was to study the repercussions of antipsychotic treatment on the synapsis in the territory of the arcuate nucleus of the hypothalamus. In that ultrastructural study, it was found that the most outstanding modifications were related with the astrocytes, whose laminae surrounded the somata and processes of the neurons, to the extent that the axosomatic synapses were

clearly reduced. This is a special case of neuroglial plasticity that tended to revert after 20 days without treatment, which could indirectly contribute to explain the frequent relapses of the patients who abandon antipsychotic treatment (if these structural modifications caused by the treatment and responsible for its actions are reversible, there is no reason to think that the manifestations of the disease will not return after treatment dropout).

This initial study encouraged us to initiate a more complete analysis, in an attempt to clarify the greater astrocyte presence occurs in other regions of the CNS that have been related with mental conditions (hippocampus, striatum, accumbens cingulate cortex, amygdala), maintaining the hypothalamus as reference. Since the electron microscope for this new approach was not very viable, we proposed performing an immunohistochemical study, using the GFAP as a marker of the astrocytes. This is a well-known protein that is usually used as a marker of this cell type and its activity. Our data show that the response is greater in the cingulate cortex and in the central part of the nucleus accumbens, ventral to the anterior white commissure. Other territories also show glial response (hypothalamus, striatum) but with lower intensity, while there are some in which differences are hardly seen (hippocampus, amygdala).

In general, these findings agree with the works of other authors who have described an increase of the glia in animals and patients treated with antipsychotics, especially with the atypical ones. Our findings show an increase in the number of astrocytes after anti-psychotic treatment, above all in the cingulate cortex and accumbens. Our samples do not make it possible to confirm if this is true in the prefrontal cortex as other authors have proposed. All of this also indicates that precaution must be taken with neuroimaging studies in schizophrenia, especially if it is not clear if the patients have been treated with antipsychotics[25].

CONCLUSIONS

1ª. The fact that antipsychotics act on the glial cells means a considerable change of our understanding on the action mechanisms of these drugs. A greater glial presence means the possibility of an improvement in functions such as neuronal nutrition, irrigation of the blood supply, supply of trophic factors, isolation of the neuronal groups or the availability of dopamine or glutamate. In conclusion, it means improvement of the synaptic regulation and, in general, of the function for the neurons of the territory[26-29]. On the other hand, it seems that the antipsychotics, both typical and atypical, through dopamine receptor blockade, may modify the production of new neurons (postnatal neurogenesis) from the stem cells in adult brains, and unexpected action that should continue to be studied[30, 31].

2ª. The corollary of this new version on the role of the glial cells and antipsychotic treatment is the change in our understanding of the CNS as a group: the neurons and glial cells maintain a continuing dialogue whose messages become a reality in substances that disseminate through the extracellular space and, among these substances, antipsychotics modulating the neuronal or glial messages, these acting as a message. These substances act by interfering with the synaptic transmission, as we already know, but they also have a role in the extrasynaptic neurotransmission (transmission volume) that involves both the neurons and the glia[32]. The new findings on extrasynaptic neurotransmission help us to understand the neuromodulation on which the CNS functions that last over time partially depend on, this depending on something more than the extremely short synaptic discharge.

3ª. It is possible that there will be an explosion of knowledge in the next few years on the glial function and the neuronal-glia dialogue which, undoubtedly, will allow us to better understand the functioning of our brain and also help us to help our patients.

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