

Review

Glial cells in neuronal network function

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Numerous evidence demonstrates that astrocytes, a type of glial cell, are integral functional elements of the synapses, responding to neuronal activity and regulating synaptic transmission and plasticity. Consequently, they are actively involved in the processing, transfer and storage of information by the nervous system, which challenges the accepted paradigm that brain function results exclusively from neuronal network activity, and suggests that nervous system function actually arises from the activity of neuron–glia networks. Most of our knowledge of the properties and physiological consequences of the bidirectional communication between astrocytes and neurons resides at cellular and molecular levels. In contrast, much less is known at higher level of complexity, i.e. networks of cells, and the actual impact of astrocytes in the neuronal network function remains largely unexplored. In the present article, we summarize the current evidence that supports the notion that astrocytes are integral components of nervous system networks and we discuss some functional properties of intercellular signalling in neuron–glia networks.

Keywords: astrocytes; intracellular Ca^{2+} ; gliotransmitter release; neuron–glia communication

1. INTRODUCTION

The brain is an assembly of cells exquisitely organized in a highly refined structure. Its function in the animal organism is to be a processor of the information received from the environment through the sensory organs as well as from its own activity, and to elaborate different biological responses that are executed by the effector organs (muscles and gland cells). The nervous system is formed by two major cell types, neurons and glial cells. Glial cells are subdivided into different types with different functions: oligodendroglia, microglia, ependimoglia and astroglia.

From the original descriptions of the cellular basis of the nervous system, neurons were promptly recognized as the main cellular elements involved in the transfer and processing of information, perhaps because they show cellular processes that extend towards sensory organs, muscles and glands. In addition, because electricity was known to be fundamental in nervous system function, the fact that neurons were electrically excitable further supported this idea. Indeed, it is well established that neuronal electrical excitability is based on the expression of numerous ligand- and voltage-gated membrane channels that give rise to membrane currents and membrane potential variations (Hille 2001), which represent the main biophysical substrate underlying the transfer and processing of information at the cellular level in the nervous system.

On the other hand, glial cells, and particularly astrocytes—the most abundant glial cell type in the central

nervous system—were considered to play simple supportive roles for neurons, probably because they lack long processes connecting sensory and effector organs. Furthermore, although astrocytes express membrane ion channels, the level of expression of some key channels is relatively low and consequently they are not electrically excitable (e.g. Orkand *et al.* 1966; Sontheimer 1994; Verkhratsky & Steinhäuser 2000; Seifert & Steinhäuser 2001). Astrocytes are known to play relevant roles in numerous processes of the development and physiology of the central nervous system, such as trophic and metabolic support for neurons, neuronal survival and differentiation, neuronal guidance, neurite outgrowth and synaptogenesis (e.g. Kettenmann & Ransom 2004). They are also key elements in brain homeostasis, regulating the local concentrations of ions and neurotransmitters (e.g. Schousboe 2003; Huang & Bergles 2004; Seifert *et al.* 2006; Wallraff *et al.* 2006), and in the control of local cerebral blood flow (e.g. Zonta *et al.* 2003; Metea & Newman 2006; Gordon *et al.* 2007; Iadecola & Nedergaard 2007). Yet, astrocytes persist in being considered passive cells providing trophic, structural and metabolic support for neurons, without actively participating in information processing by the nervous system.

However, evidence obtained during the past few years has challenged the concept of astrocytes as passive elements that simply provide adequate environmental conditions for appropriate neuronal function, and instead indicated that astrocytes play an active role as information processors in the function of the brain. In the present review article, we shall present a brief summary of the currently available data that has challenged the classical idea that brain function results exclusively from neuronal activity, and we shall discuss the available as well as

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the still required evidence that suggests brain function might actually arise from the coherent activity of neuron–glia networks.

2. ASTROCYTES SHAPE THE EXTRACELLULAR SPACE STRUCTURALLY AND FUNCTIONALLY

Astrocytes are known to play important roles in the homeostasis of the extracellular environment, providing the adequate conditions for the appropriate function of neurons and synapses. Because astrocytic processes are highly dynamic subcellular elements capable of mobility, retraction and extension, astrocytes can dynamically shape the extracellular space, which may have a strong impact on the neuronal network by influencing the extracellular diffusion of neurotransmitters (Syková & Nicholson 2008; Theodosis *et al.* 2008). The hypothalamic supraoptic nucleus has been a paradigmatic brain area where activity-dependent structural changes in the astrocytic coverage and the consequent functional changes have been clearly identified (for a review see Theodosis *et al.* (2008)). Modifications of the astrocytic ensheathing of synapses that occur under specific physiological conditions strongly influence synaptic efficacy, owing to changes in the effectiveness of glutamate clearance (Oliet *et al.* 2001) and in the effective extracellular levels of the gliotransmitter D-serine, which modulates *N*-methyl-D-aspartate (NMDA) receptor-mediated synaptic transmission (Panatier *et al.* 2006).

Hippocampal astrocytes have also been shown to rapidly extend and retract their processes in coordination with changes in dendritic spines, which may lead to the consolidation of dendritic spines (Haber *et al.* 2006). Likewise, changes in the coverage of synapses as well as in the expression of glutamate transporters by astrocytes in the barrel cortex have been shown to occur after whisker stimulation (Genoud *et al.* 2006). However, the actual functional impact on synaptic physiology in hippocampus and cortex, respectively, remains unknown.

In addition to this structural constraint imposed by astrocytes, the extracellular space can be also functionally delimited by the polarized expression of membrane proteins such as voltage-gated channels and neurotransmitter transporters, which may create extracellular pathways and spatial restrictions for the diffusion of ions and transmitters. Absence or malfunction of these membrane proteins affects neuronal network activity and leads to important nervous system dysfunctions. For instance, astrocytes control the extracellular K^+ concentration through the expression of specific ion channels, such as inward-rectifying K^+ channels. The impairment of K^+ buffering by a reduced expression of these channels has been proposed to contribute to neuronal hyperexcitability and epilepsy (Seifert *et al.* 2006). Likewise, expression of glutamate transporters by astrocytes has been shown to be crucial in the clearance of glutamate from the synaptic cleft to terminate synaptic function (Tzingounis & Wadiche 2007). Furthermore, transmitter transporter dysfunctions have been shown to lead to several pathological conditions, including hyperexcitability of

the neuronal networks and the development of epileptic activity (Maragakis & Rothstein 2004).

These examples show relevant consequences that the structural and functional limits imposed by astrocytes may have on neuronal network function, such as controlling neuronal hyperexcitability and epileptic activity. Furthermore, these constraints may also have more subtle but highly relevant impacts on network function because they may grant a point-to-point communication between neurons, or alternatively allow a more diffuse action of neurotransmitters that may then act on different cellular targets through neurotransmitter spillover (Rusakov 2001).

3. ASTROCYTES ARE COMMUNICATING CELLS

Because neuronal function mainly relays electrical signals and astrocytes are not electrically excitable cells, they have been largely neglected as signalling cells when considering neuronal network function. However, the use of Ca^{2+} -sensitive fluorescent dyes that monitor intracellular levels of ions in living cells has demonstrated that astrocytes display a form of cellular excitability based on variations of the Ca^{2+} concentration in the cytosol rather than electrical changes in the membrane (Cornell-Bell *et al.* 1990; Charles *et al.* 1991; for a review see Perea & Araque (2005a)). Neurons use the electrochemical gradients across the plasma membrane to generate electrical signals because of the high expression of specific ligand- and voltage-gated ion channels (Hille 2001). Voltage-gated channels expressed by astrocytes mainly function as a mechanism for the homeostatic maintenance of the extracellular levels of ions (Seifert *et al.* 2006), with minor, if any, implications for cellular signalling. In contrast, astrocytes mainly use the Ca^{2+} stored in the endoplasmic reticulum as the source for cytoplasmic Ca^{2+} , which acts as a cellular signal (Perea & Araque 2005a). Therefore, while neurons base their cellular excitability on electrical signals generated across the membrane, astrocytes base their cellular excitability on variations of the Ca^{2+} concentration in the cytosol. These Ca^{2+} variations may serve as an intracellular and intercellular signal that can propagate within and between astrocytes, signalling to different regions of the cell and to different cells (Perea & Araque 2005a). As detailed below, this calcium signal may stimulate different responses with important implications for neuronal function.

4. ASTROCYTES ARE AN INTEGRAL PART OF THE SYNAPSE: TRIPARTITE SYNAPSES

For a long time, intercellular signalling underlying transfer and processing in the brain was considered to occur exclusively between neurons. Numerous studies performed during the past few years, have established the existence of bidirectional signalling between neurons and astrocytes. The calcium-based cellular excitability displayed by astrocytes can be triggered by neuronal and synaptic activity through activation of neurotransmitter receptors expressed by astrocytes (Perea & Araque 2005a). In turn, astrocyte calcium elevations stimulate the release of different

neuroactive substances—called gliotransmitters—such as glutamate, ATP and D-serine, which regulate neuronal excitability and synaptic transmission (Haydon & Araque 2002; Volterra & Bezzi 2002; Perea *et al.* 2009). These findings have led to the establishment of a new concept in synaptic physiology, the tripartite synapse, in which astrocytes exchange information with the neuronal synaptic elements (Araque *et al.* 1999). Consequently, astrocytes are an integral part of the synapses, being involved not only in passive homeostatic control of adequate conditions for synaptic function, but also actively in synaptic function (Perea *et al.* 2009).

5. ASTROCYTES PROCESS SYNAPTIC INFORMATION

A typical neuron in the mammalian central nervous system may in contact with thousands of synaptic terminals, and consequently may receive a large wealth of synaptic information that must integrate and be processed to elaborate a single output signal in the form of action potentials conveyed by the axon. Such integration is based on functional properties of neurons, including the selective responsiveness to different input signals and the existence of neuronal intrinsic properties that account for the nonlinear input–output relationship (Llinas & Sugimori 1980; Agmon-Snir *et al.* 1998).

Recent findings show that astrocytes do not simply respond passively to synapses performing a linear readout of the synaptic activity level. Rather, the demonstration that astrocytes display selective responsiveness to specific synaptic inputs, show a calcium-based excitability that displays nonlinear input–output relationships, and have intrinsic properties that grant this nonlinearity (for a review see Araque 2008), indicates that neuron-to-astrocyte communication presents properties of complex information processing that are classically considered to be exclusive to neuron-to-neuron communication. It also indicates that astrocytes can be considered as cellular processors of synaptic information (Perea *et al.* 2009).

6. ASTROCYTES ARE AN INTEGRAL PART OF NEURAL NETWORKS: NEURON–GLIA NETWORKS

Once it is realized that astrocytes are able to release gliotransmitters which regulate neuronal excitability and synaptic transmission and plasticity, it is immediately obvious that they may strongly affect neural network function. The relevance of glia to neuronal network function has been demonstrated on paradigmatic neural networks, such as brainstem respiratory (Hülsmann *et al.* 2000) and spinal cord locomotor (Baudoux & Parker 2008) networks, where disruption of glial function by gliotoxins strongly affects rhythmic network activity. These effects cannot be exclusively accounted for by the metabolic contribution of glial cells to neuronal network function, which suggests an active role of glia in neuronal network activity (Baudoux & Parker 2008). The detailed cellular signalling mechanisms involved in astrocytic influence

on neuronal network activity are largely unknown. Several findings suggest that this influence does not consist of simple regulation of neuronal membrane potential through activation of postsynaptic receptors—such as NMDA receptor-mediated slow inward currents (SICs) or gamma-aminobutyric acid (GABA) receptor-mediated slow outward currents evoked by glutamate or GABA released from astrocytes, respectively (Angulo *et al.* 2004; Fellin *et al.* 2004; Perea & Araque 2005b; Kozlov *et al.* 2006; Navarrete & Araque 2008)—or modulation of synaptic strength by pre- or postsynaptic mechanisms (for a review see Perea *et al.* 2009). These simple individual events may result in complex signalling phenomena when considering the fine details and the variety of molecular mechanisms underlying astrocyte–neuron communication. In the following paragraphs, we shall present some examples of our current knowledge on the physiological consequences of astrocyte–neuron signalling, which simply provides a mere glimpse of the actual complexity that astrocytes may introduce in the neuronal network function.

Because a single astrocyte may be in contact with thousands of synapses, and astrocytes can communicate between them, perhaps the simplest effect of astrocytes on neuronal networks is their ability to simultaneously activate populations of neurons. This effect has been demonstrated in the hippocampus, where astrocyte calcium elevations and subsequent glutamate release lead to the synchronous excitation of clusters of pyramidal neurons, indicating that gliotransmission may contribute to neuronal synchronization (Angulo *et al.* 2004; Fellin *et al.* 2009).

A single gliotransmitter may exert multiple effects on the neuronal network. For example, in the hippocampus, glutamate released from astrocytes may (i) evoke SICs in CA1 hippocampal pyramidal neurons through activation of postsynaptic NMDA receptors (Angulo *et al.* 2004; Fellin *et al.* 2004; Perea & Araque 2005b; Navarrete & Araque 2008); (ii) enhance synaptic strength through activation of presynaptic group I metabotropic glutamate receptors in CA3–CA1 synapses (Fiacco & McCarthy 2004; Perea & Araque 2007); (iii) facilitate synaptic transmission through activation of presynaptic kainate receptors in interneurons (Liu *et al.* 2004a); (iv) increase neurotransmission through activation of presynaptic NMDA receptors in dentate granule cells (Jourdain *et al.* 2007); (v) modulate inhibitory synaptic transmission between interneurons and CA1 pyramidal cells (Kang *et al.* 1998); (vi) depress inhibitory transmission through activation of group II/III metabotropic glutamate receptors (Liu *et al.* 2004b). The simultaneous or differential expression of these effects at individual cells may strongly affect hippocampal function.

The general principle extracted from these examples is that a single gliotransmitter may have different effects depending on the target neurons and neuronal elements (pre- or postsynaptic) as well as the activated receptor subtypes, which provides a high degree of complexity for astrocytic effects on neuron–glia network activity.

But even more complexity arises from the fact that astrocytes may release different gliotransmitters, such

as glutamate, ATP, GABA or D-serine, with different potential neuromodulatory roles (for a review see Perea *et al.* 2009). In the olfactory bulb, astrocytes have been shown to release GABA and glutamate, leading to a target cell-specific modulation of neuronal activity, because astrocytic GABA inhibits mitral and granule cells, whereas astrocytic glutamate excites granule cells (Kozlov *et al.* 2006). We still do not know whether different gliotransmitters are co-released, released by different astrocytes or by different astrocytic processes. We do not know either the specific incoming inputs or the physiological conditions that govern the precise release of each gliotransmitter, but it would not be surprising if specific signals control gliotransmitter release, as was recently demonstrated in hippocampal slices, where astrocyte Ca^{2+} elevations induced by activation of PAR-1 receptors, but not P2Y1 receptors, stimulates glutamate release to evoke NMDA receptor-mediated SICs in pyramidal neurons (Shigetomi *et al.* 2008). Neither do we know what could be the possible emergent consequences on neuronal network function if several different gliotransmitters act cooperatively on the same neuron or act on different cell types evoking diverse responses, but we can easily envisage the high degree of complexity that astrocytes may consequently introduce in neuronal network activity.

On the other hand, astrocytes may expand the possible consequences of a single neurotransmitter on network function. Indeed, in the CA1 area of the hippocampus, glutamate released from Schaffer collaterals excites postsynaptic pyramidal neurons as well as inhibitory interneurons, which in turn activate astrocytes elevating their intracellular Ca^{2+} . This astrocyte Ca^{2+} signal stimulates the release of ATP, which after being extracellularly degraded to adenosine leads to synaptic depression of adjacent excitatory synapses (Zhang *et al.* 2003; Serrano *et al.* 2006). Therefore, this heterosynaptic depression of synaptic transmission is mediated by the coordinated activation of successive intercellular signalling events (i.e. excitatory axons stimulate interneuron activity that triggers a GABA-mediated astrocyte Ca^{2+} signal that, in turn, stimulates release of ATP that is converted to adenosine to depress synaptic transmission) and represents a simple but elegant example of the emergent consequences provided by astrocyte activity on neuronal network signalling.

Another example of the emergent signalling provided by astrocytes has been recently reported in the hippocampus, where astrocytic glutamate released in response to endocannabinoids released by a pyramidal neuron can act as an extracellular messenger that stimulates adjacent neurons (Navarrete & Araque 2008). Hence, astrocytes may influence neuronal network activity by providing a bridge for non-synaptic communication between neurons.

Astrocytes may also transform the signalling sign of a neurotransmitter. Endocannabinoids released by neurons are known to retrogradely suppress synaptic transmission through activation of presynaptic cannabinoid receptors (Chevalleyre *et al.* 2006). However, we have found that these endocannabinoids may lead to heterosynaptic enhancement of synaptic strength

of distant synapses through stimulation of astrocytes (M. Navarrete & A. Araque 2010, unpublished data).

Finally, although the neuromodulatory roles of astrocytes *in vivo* are still largely undefined, recent studies have begun to investigate such roles on complex brain activity and animal behaviour. Adenosine metabolized from ATP released by astrocytes has been shown to participate in the accumulation of sleep pressure and to contribute to cognitive deficits associated with sleep loss (Halassa *et al.* 2009). The gliotransmitters D-serine and ATP/adenosine have been shown to be involved in slow cortical oscillations *in vivo* (Fellin *et al.* 2009). These studies not only demonstrate that astrocyte–neuron interaction occurs *in vivo*, but also show that astrocytes may influence the output of complex neuronal networks.

7. FUTURE QUESTIONS

Neuron–glia interaction has been analysed mainly at the cellular and subcellular levels. Although much work is still required to elucidate key questions at these basic levels (Perea *et al.* 2009), cell physiology studies need to pursue a better understanding of basic molecular and single-cell phenomena. In comparison, the actual impact of glial cells on the physiology of defined neuronal network activity remains unknown. Likewise, scarce data exist on animal behaviour or complex network activity. Using transgenic mice, recent studies have shown the involvement of gliotransmission in cognitive deficits associated with sleep loss and in slow cortical oscillations (Fellin *et al.* 2009; Halassa *et al.* 2009). While the underlying molecular and cellular mechanisms are defined by the experimental approach, i.e. transgenic mice with loss of function of gliotransmitter release, it remains unknown how the neuron–glial network activity that gives rise to these brain and behavioural states is affected.

Imaging of astrocyte calcium levels was the initial experimental step of the ‘glial revolution’, revealing unexpected properties of astrocytes that prompted the re-evaluation of their role in neurophysiology (for reviews see Haydon (2002) and Perea *et al.* (2009)). The calcium signal is the key element in the bidirectional communication between astrocytes and neurons, because it represents the basis of the astrocytic cellular excitability modulated by the synaptic activity, and is responsible for the Ca^{2+} -dependent release of gliotransmitters (Perea *et al.* 2009). Yet, alternative technologies aimed at monitoring other astrocytic intracellular signals (such as IP₃, cGMP and cAMP) may contribute to give more insights, and even to reveal novel intracellular and intercellular signalling mechanisms and consequences of astrocyte function and its impact on neuronal network activity.

Cell biology studies have begun to reveal that astrocytes are not a single cellular entity, but rather the term astrocyte likely comprises a wide variety of cellular subtypes (Kettenmann & Ransom 2004). Just like neurons, these cellular subtypes may differ not only phenotypically but also functionally, and their functional role may probably depend on the neuron–glia network in which they are immersed. A thorough

characterization of the phenotypic as well as functional properties of astrocytes within and across different brain areas is required. For instance, assuming that glutamate-mediated signalling from astrocytes to neurons is a widespread phenomenon, in different brain regions it obviously may have different physiological meaning in neuronal network function.

To begin to define precisely the actual role of astrocytes in neuron–glia networks, might require the issue to be analysed in well-defined simple neuronal networks. Because of their relative simplicity, invertebrate system studies have proved to be extremely useful in elucidating many aspects of neurophysiology and neuronal network function. Although invertebrate and vertebrate glial cells may share similar features (Freeman & Doherty 2006; Lohr & Deitmer 2006; Shaham 2006; Murai & Van Meyel 2007; Jackson & Haydon 2008), the phylogenetic differences between vertebrates and invertebrates of the phenotype and function of glial cells may make it difficult to extrapolate and generalize the conclusions obtained in invertebrate systems. Additionally, glial cells are more numerous, display higher morphological complexity and probably have more complex functions in mammals (Ramón y Cajal 1899; Nedergaard *et al.* 2003).

Two main experimental strategies can be devised to study the impact of astrocyte–neuron signalling in neuronal network activity, namely (i) selective stimulation of astrocytes and (ii) specific loss of function of astrocyte activity that does not compromise non-signalling glial function involved in the homeostasis and metabolic support for neurons. The development of transgenic animal models will be useful for these purposes, but only if appropriate controls are taken to guarantee the proper spatio-temporal expression of exogenous receptors as well as appropriate coupling to intracellular signalling cascades, and cellular events such as gliotransmitter release are functionally preserved (for a discussion see Perea *et al.* 2009).

Selective stimulation of astrocytes is one of the major experimental limitations encountered when investigating the role of astrocytes in neuronal network function. Astrocytes express a wide variety of transmitter receptors that upon activation trigger the astrocyte Ca^{2+} signal. However, such receptors are also present in neurons, therefore making it difficult unequivocally to ascribe any possible effect to astrocyte activity. Specific transgenic expression in astrocytes of exogenous receptors that are not expressed endogenously in the brain has provided discouraging negative results (Fiacco *et al.* 2007; for discussions see Agulhon *et al.* (2008) and Perea *et al.* (2009)). Transgenic expression of alternative receptors needs to be assayed to settle the issue. For instance, the specific expression by astrocytes of fast light-responsive microbial opsin such as channelrhodopsin-2 (Gradinaru *et al.* 2009), which would allow the fast and cell-specific stimulation of astrocytes, might be a promising tool.

Transgenic animals displaying loss of function in astrocytic activity have been shown to be useful. The use of transgenic mice in which SNARE-dependent release of gliotransmitters from astrocytes was abolished has allowed demonstration of the involvement of glial cells in specific brain activities and

animal behaviours (Fellin *et al.* 2009; Halassa *et al.* 2009). These studies aimed to investigate the consequences of astrocytic gliotransmission at a high-level network output of the system. Analysis of the effects at a lower level of complexity, i.e. neuronal network activity, is still required and may provide important insights on how the coordinated activity of the neural circuit may be affected by astrocytes.

8. CONCLUDING REMARKS

As discussed in the introductory article of this issue, understanding neuronal networks has been revealed as an extremely challenging task beset with multiple conceptual and technical limitations. These limitations seem to be exponentially amplified when trying to understand neuron–glial networks, because the novelty of the concept (and consequently the scarce experimental data available) and the complexity that glial cells introduce to the system. When comparing pure neuronal networks with neuron–glia networks, not only are the number of cells involved increased, but the possible connectivity and signalling processes are also increased by orders of magnitude. Furthermore, the rules governing intercellular signalling in the network function (e.g. electrical and calcium-based excitability; neurotransmission and gliotransmission) are also increased, not only quantitatively but also qualitatively. Just as astrocytes endow the system with higher complexity and higher degrees of freedom, they also make the task of understanding neuron–glia networks more complex.

Considering a pure neuronal network, several elements define that network function, such as cellular type (i.e. excitatory or inhibitory), neuronal excitability, cell-to-cell connectivity and neurotransmission. Our current knowledge of astrocytes and astrocyte–neuron interaction indicates that several facts need to be considered to gain a complete understanding of the actual structure and function of the network: (i) besides neurons, astrocytes represent an additional cellular type integrated in the network; (ii) besides electrical excitability of neurons, there is a calcium-based cellular excitability of astrocytes; (iii) besides neuron–neuron connectivity there is astrocyte–astrocyte connectivity as well as astrocyte–neuron connectivity; (iv) besides neurotransmission, gliotransmission also plays an important role in network function.

Abundant findings indicate that astrocytes can no longer be neglected when considering intercellular signalling underlying information processing and neuronal network activity. Indeed, astrocytes integrate and process synaptic information, and in turn modulate neuronal excitability as well as synaptic transmission and plasticity. The corollary of the concept of the tripartite synapse, i.e. astrocytes are integral components of the neuronal networks, and therefore nervous system function results from the activity of neuron–glial networks, seems to flow logically from these findings. However, experimental evidence is still scarce and conceptual interpretations are still at a very early stage. Nevertheless, we have gained enough knowledge to realize the importance of glial cells, and we have probably reached a position

that prompts us to re-evaluate neuronal network properties and functions to include astrocytes. Exhaustive studies are required to elucidate the actual details and implications of incorporating glial cells into neuronal networks to become neuron–glial networks. We definitely have a long way to go in order to understand neuron–glia networks, but at least we have made the first step in realizing what are the actual elements involved in the function of the nervous system networks.

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