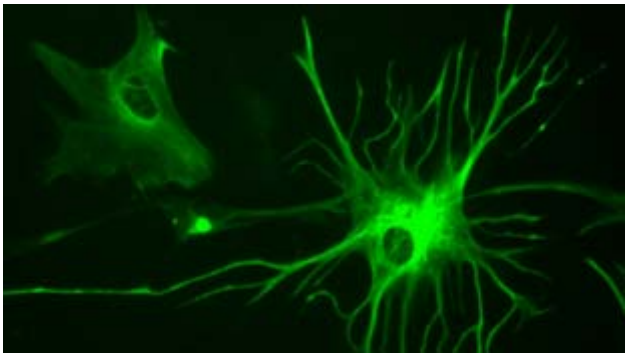
Mice Learn Faster with Human Glia

Mice with human brain cells showed enhanced synaptic plasticity and learning, suggesting glia may be key to our cognitive prowess.

March 7, 2013



Mice that received transplants of human glial progenitor cells learned much more quickly than normal mice, according to a study published today (March 7) in *Cell Stem Cell*. The findings support the theory that glial cells made a significant contribution to the evolution of our own enhanced cognitive abilities.

“This work is very exciting and surprising because it demonstrates that there may be something special about human glial progenitor cells that contribute to the amazing complexity and computational abilities of the human brain,” said Robert Malenka, a neuroscientist at Stanford University who was not involved in the study, in an email to *The Scientist*.

For many years, glia cells, non-neuronal cells present in the same numbers as neurons in the brain, were thought to play only a supporting role, providing structure, insulation, and nutrients for neurons. But in the past 20 years it has become clear that glia also participate in the transmission of electrical signals. Specifically, astrocytes—a type of glial cell with thousands of tendrils that reach and encase synapses—can modulate signals passing between neurons and affect the strength of those connections over time.

Recent studies have also demonstrated that human astrocytes are very different from those found in mouse and rat brains, on which most previous studies of astrocyte physiology were based. Human astrocytes are more numerous, larger, and more complex, and they are capable of far more rapid signaling responses than rodent astrocytes.

Together, these results suggest that astrocytes may have been critical to the evolution of enhanced neural processing in humans. Having already transplanted human glial progenitor cells (GPCs) to restore myelination in myelin-deficient mice, Steven Goldman of University of Rochester Medical Center in New York and colleagues realized that they could repeat the trick in normal mice to assess the contribution of human-specific astrocytes to synaptic plasticity and learning.

Goldman's team grafted human GPCs into the brain of baby mice and waited until they became adults, by which time a large proportion of their forebrain glia were replaced by human cells differentiated from the GPCs, including astrocytes with the same structure and functional capabilities as in humans. The researchers then looked at long-term potentiation (LTP)—the strengthening of synaptic connections and a key mechanism underlying learning—in the hippocampus, and found that it was significantly enhanced in mice with human GPCs compared with normal mice and mice engrafted with mouse GPCs. Goldman and colleagues also assessed the performance of the mice on several behavioral tasks that measure leaning and memory—including auditory fear conditioning, a maze test, and object-location memory—and found across the board that mice with human GPCs learned significantly more quickly than normal mice.

"The most remarkable result is that the human glial progenitor cells implanted in the mouse brain caused enhanced learning," said Malenka. "I don't think most neuroscientists, including those that focus on glia, would have predicted that result."

Exploring potential molecular mechanisms behind such an effect, Goldman and his colleagues noticed significant increases in the release of a cytokine called TNFa in the human-derived astrocytes in mice. And when they blocked the production of this molecule, LTP was reduced, as was performance in the object-location memory task, suggesting that TNFa is an important player in the glia-mediated enhancement of synaptic function.

For Malenka, the study offers "further evidence for the increasingly accepted idea that glial cells importantly influence synaptic function." Goldman went further, adding that the results provide "a substantial clue" to the basis of human smartness. "We now have to view the evolution of glial form and function as one of the most important aspects of human cognitive evolution," said Goldman.

Furthermore, having reported in February that they can generate human GPCs from skin cells reprogrammed into induced pluripotent stem cells, Goldman's team can now make patient-specific GPCs from individuals with neuropsychiatric and neurological diseases thought to be specific to humans or primates—and therefore potentially glial-associated. By implanting these cells into normal mice, the researchers can create in vivo models with which to investigate the role of glial cells in these disorders.

"This gives us a broad platform to sort out the differential contribution of glia and neurons in certain diseases," said Goldman, who is already analyzing data from such experiments on schizophrenia and Huntington's disease. "Most current therapeutics target neurons, but this gives us the potential to develop glial targets for new drugs."

X. Han et al., "Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity and learning in adult mice," *Cell Stem Cell*, 12:342-53, 2013.

Sinapsis tripartita

Durante decenios se supuso que la neuroglía desempeñaba una simple función de soporte de las neuronas. Nuevos hallazgos indican que las células de glía intervienen de una manera activa en el procesamiento cerebral de la información

Gertrudis Perea y Alfonso Araque

La máquina más poderosa, capaz de cruzar océanos, ver más allá de Orión, viajar en el tiempo y comprender el universo —nuestro cerebro—, no es sino un conjunto exquisitamente organizado de células, cuya función consiste en comportarse como un procesador de la información que recibe del medio y de su propia actividad y en elaborar diferentes respuestas biológicas. Consta de dos grandes tipos de células: las neuronas y las células gliales o neuroglía.

Desde la primera descripción de las células gliales por Rudolf Virchow en 1846, la función original atribuida a la neuroglía fue la de ser el aglutinante (el significado en griego de glía) del sistema nervioso (SN). La investigación posterior subdividió las células gliales en varios grupos: oligodendrocitos y células de Schwann (responsables de la formación de la vaina de mielina que envuelve los axones neuronales en el sistema nervioso central y periférico, respectivamente), microglía (con funciones fagocíticas implicadas en procesos inflamatorios) y astrocitos, el subtipo glial más abundante en el SNC. Sobre los astrocitos centraremos nuestra exposición.

Desde los primeros estudios del SN, las neuronas se consideraron los elementos celulares responsables de la elaboración y transmisión de información. A ello contribuyó la aceptación de que la actividad nerviosa tenía un sustrato eléctrico y

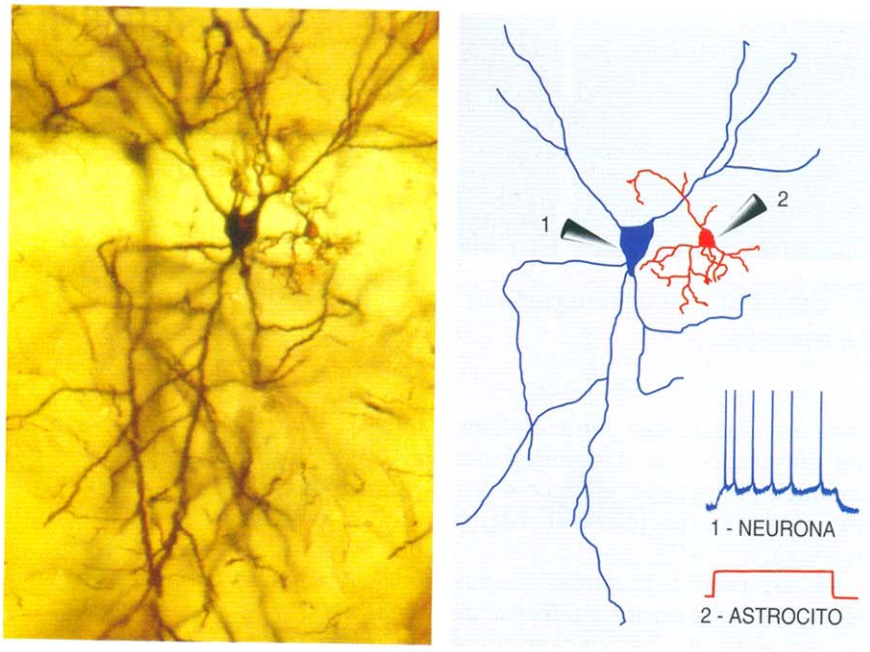
TODAS LAS ILUSTRACIONES DE ESTE ARTICULO: CORTESIA DEL AUTOR

1. 'NEUROGLIA DE LA CAPA DE LAS PIRAMIDES y estrato radiado del asta de Ammón. Hombre adulto autopsiado tres horas después de la muerte. Cloruro de Oro'. Dibujo original de S. Ramón y Cajal (1899). Original depositado en el Instituto Cajal. CSIC. Madrid.

que las neuronas eran células eléctricamente excitables. A la neuroglía se le reservaba la misión única de soporte trófico y estructural de las neuronas.

Frente a la teoría del relleno de la función glial, imperante a finales del siglo XIX, Santiago Ramón y Cajal contrapuso en 1899 la teoría del aislamiento, que concedía un papel activo a los astrocitos en la fisiología neuronal:

> *"El prejuicio de que las fibrillas neuróglicas son a las células nerviosas lo que los haces colágenos del tejido conectivo a los corpúsculos musculares o glandulares, es decir, una trama pasiva de mero relleno y sostén (y cuando más, una ganga destinada a embeberse en jugos nutritivos), constituye sin duda el principal obstáculo que el observador necesita remover para formarse un concepto racional de la actividad de los corpúsculos neuróglicos".*



2. MICROGRAFIA generosamente cedida por Laura López Mascaraque (Instituto Cajal, Madrid), que muestra una neurona y un astrocito de hipocampo. A la derecha, neuronas y astrocitos presentan distinto comportamiento eléctrico. Mientras que las neuronas generan potenciales de acción, los astrocitos responden pasivamente a estímulos que despolarizan la membrana celular.

Sin embargo, ante la falta de herramientas y técnicas experimentales que aportaran datos sobre la auténtica función de los astrocitos, la idea de una función pasiva de la glía persistió en sus términos esenciales durante más de cien años.

En el último siglo, la función de los astrocitos se ha ampliado notablemente. Sabemos ya que desempeñan una función importante en numerosos aspectos del desarrollo, el metabolismo y la patología del sistema nervioso. Resultan decisivos, por ejemplo, en el soporte trófico y metabólico de las neuronas, la supervivencia, diferenciación y guía neuronal, la sinaptogénesis y la homeostasis cerebral. Ello no impedía que los astrocitos continuaran considerándose meras células de soporte trófico, estructural y metabólico de las neuronas, sin participación activa en el procesamiento y elaboración de información por el SN.

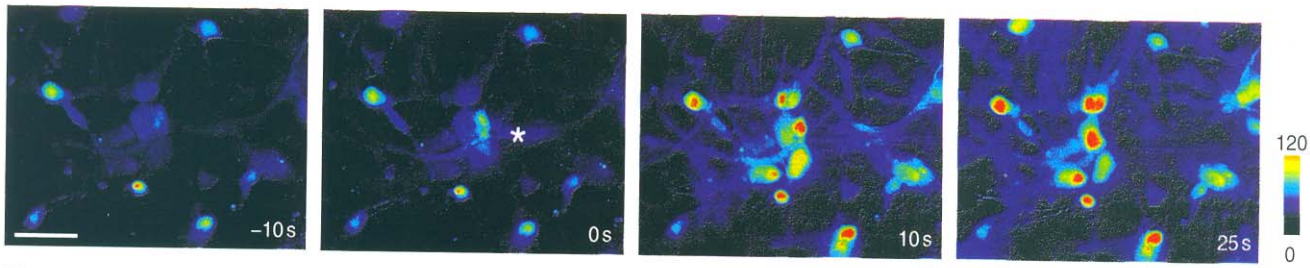
Hubo que esperar a los años noventa del pasado siglo, al advenimiento de nuevas herramientas de estudio, para percatarse de la función crítica desempeñada por las células gliales. Las nuevas investigaciones se basan en técnicas y aproximaciones experimentales que tienen por objeto dilucidar los procesos y mecanismos fisiológicos responsables del funcionamiento de entidades orgánicas: células, tejidos u organismos. Tales herramientas que podemos englobar bajo el término fisiológica, se aplican a distintos niveles de complejidad (celular, sistémica o conductual).

Entre las nuevas técnicas se enumeran registros eléctricos en células individuales ("patch-clamp"), técnicas microscópicas novedosas (epifluorescencia para la imagen de los niveles intracelulares de iones, microscopía confocal y de dos fotones, fotoestimulación con luz ultravioleta de compuestos enjaulados), técnicas de imagen cerebral *in vivo*, etcétera. Gracias a tales métodos de análisis, podemos ratificar la vigencia, 100 años después, de la idea expresada en 1899 por Cajal. Repasaremos aquí las pruebas experimentales que respaldan la existencia de comunicación entre astrocitos y neuronas, y, por tanto, la participación activa de los astrocitos en el funcionamiento del sistema nervioso.

El ion calcio

A las células gliales se les negaba una participación activa en la fisiología del sistema nervioso porque carecían de la propiedad fundamental que poseían las neuronas, a saber, la excitabilidad eléctrica o capacidad de sufrir cambios en su potencial de membrana. Las variaciones del potencial de la membrana celular constituían el sustrato biofísico de la codificación de información neuronal y servían de base para los procesos celulares de transferencia de información en las neuronas. Las células gliales, en cambio, eran células inexcitables eléctricamente, que apenas presentaban pequeñas variaciones en su potencial de membrana. ¿Era cierto? No exactamente.

El desarrollo de nuevas técnicas de imagen permitió, a principios de los noventa, poner de manifiesto que los astrocitos, considerados hasta entonces células pasivas, evidenciaban una excitabilidad celular, que se apo-



3. GENERACION y propagación de una onda de calcio en astrocitos del hipocampo.

yaba en variaciones intracelulares del ion calcio. Ese descubrimiento revolucionó nuestra visión de los astrocitos y su función en el sistema nervioso.

La excitabilidad celular de los astrocitos se presenta en forma de un aumento de la concentración citoplasmática de calcio. Actúa ese incremento a modo de señal intracelular, desencadenante de diversas respuestas celulares. La señalización por calcio puede presentarse espontánea o en respuesta a diferentes estímulos. Según veremos más adelante, resulta crucial que tal excitabilidad se desencadene por la actividad de las neuronas.

La señal de calcio puede propagarse en el interior celular (señal intracelular) o propagarse en diferentes astrocitos (señal intercelular con importantes consecuencias funcionales). Quedó patente la comunicación entre astrocitos a través de cultivos de los mismos, donde se demostró que los aumentos de calcio originados en una célula podían propagarse a las células vecinas y generar una "onda de calcio". Las ondas de calcio creadas representarían una nueva forma de comunicación intercelular a larga distancia en el sistema nervioso.

La sinapsis tripartita

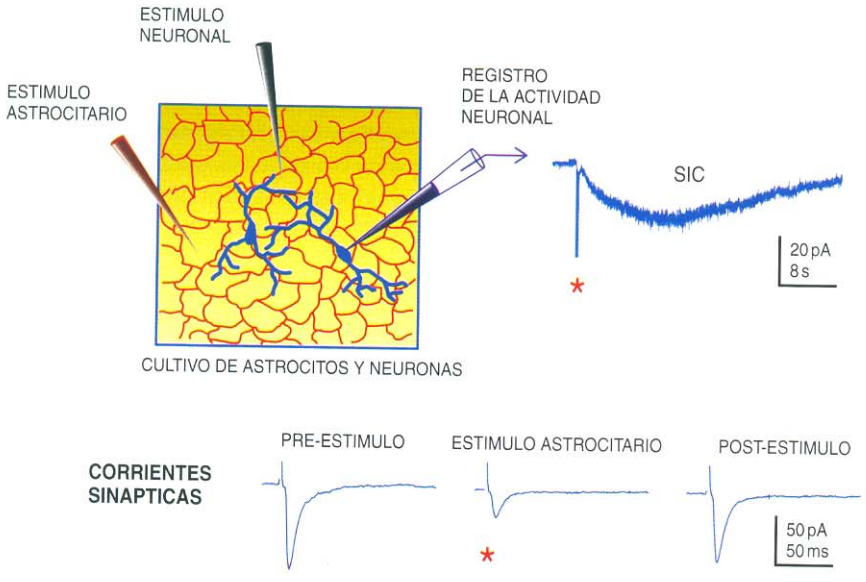
A lo largo del SNC existe una íntima asociación entre las expansiones celulares de los astrocitos (procesos astrocitarios) y los elementos neuronales, sobre todo en las sinapsis, estructuras donde se produce la transferencia de información entre neuronas. La comunicación entre neuronas se produce mediante la liberación de neurotransmisores químicos desde la neurona presináptica, que provocan la activación de receptores específicos en la neurona postsináptica, con la consiguiente generación de respuestas eléctricas. El proceso de neurotransmisión descrito representa la principal forma de comunicación en el SN.

La estrecha relación física entre neuronas y astrocitos entraña una adecuada disposición para su interacción funcional. Estudios llevados a cabo en distintas áreas cerebrales han demostrado que los neurotransmisores liberados por las terminales sinápticas pueden activar los receptores presentes en la membrana de los astrocitos, que desencadenan una señal de calcio astrocitaria. En otras palabras, existe una comunicación entre neuronas y astrocitos.

El aumento de calcio intracelular en astrocitos puede dar lugar a diferentes respuestas celulares, entre las que destaca la liberación de gliotransmisores. De notable interés fisiológico, estas moléculas transmisoras, segregadas por células gliales, pueden modular la excitabilidad neuronal y la transmisión sináptica. De ese modo, los astrocitos no sólo responden a la actividad neuronal, sino que pueden también enviar señales de comunicación a las neuronas.

De la existencia de comunicación bidireccional entre astrocitos y neuronas ha surgido un nuevo concepto en la fisiología sináptica: la sinapsis tripartita. En razón de la misma, la sinapsis constaría de tres elementos, a saber, los elementos pre y postsinápticos neuronales y los astrocitos adyacentes. En la sinapsis tripartita los astrocitos desarrollan funciones activas como elementos reguladores en la transferencia de información en el sistema nervioso.

4. LOS ASTROCITOS modulan la actividad eléctrica neuronal y transmisión sináptica.



Los astrocitos procesan la información sináptica

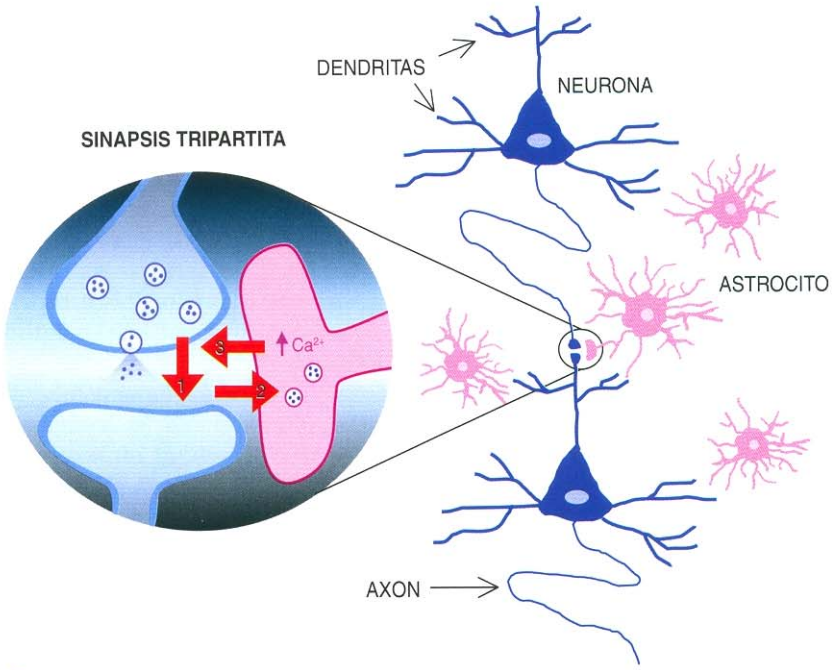
Entenderemos la naturaleza de la comunicación entre neuronas y astrocitos si tenemos en cuenta que los astrocitos expresan en su membrana un amplio repertorio de receptores de distintos transmisores, cuya activación genera aumentos del calcio intracelular en los astrocitos. Ese incremento del ion les permite a los astrocitos responder a diferentes neurotransmisores liberados por las neuronas en distintas regiones del cerebro.

Abundan las pruebas sobre la respuesta de los astrocitos a los neurotransmisores glutamato, GABA, acetilcolina, noradrenalina y óxido nítrico. Queda, sin embargo, por determinar el control que otros sistemas de neurotransmisores puedan ejercer sobre la excitabilidad de los astrocitos.

Se admite, desde hace tiempo y sin discusión alguna, que las neuronas constituyen los elementos celulares responsables del procesamiento de información en el sistema nervioso. Una característica fundamental de las neuronas, relacionada con el procesamiento de información, estriba en su capacidad de integrar la información procedente de múltiples sinapsis. Nos referimos al proceso en cuya virtud las neuronas, gracias a las propiedades intrínsecas de su membrana, se hallan capacitadas para sumar distintas entradas excitadoras e inhibidoras y elaborar una respuesta en función de ellas.

Hemos visto que los astrocitos responden con aumentos de calcio a la actividad neuronal. ¿Presenta la comunicación entre neuronas y astrocitos propiedades complejas características de la comunicación entre neuronas? En otras palabras, ¿pueden los astrocitos modular su señal de calcio, la base de su excitabilidad celular, en respuesta a distintas sinapsis? ¿Responden, por el contrario, de forma pasiva a la actividad neuronal?

En nuestro laboratorio del Instituto Cajal hemos abordado la cuestión. Investigamos las propiedades de la señal de calcio astrocitaria en respuesta a la estimulación de dos tipos de sinapsis que liberan diferentes



5. SINAPSIS TRIPARTITA. Además del flujo de información entre los elementos neuronales (1), existe un flujo de información bidireccional entre neuronas y astrocitos (2, 3).

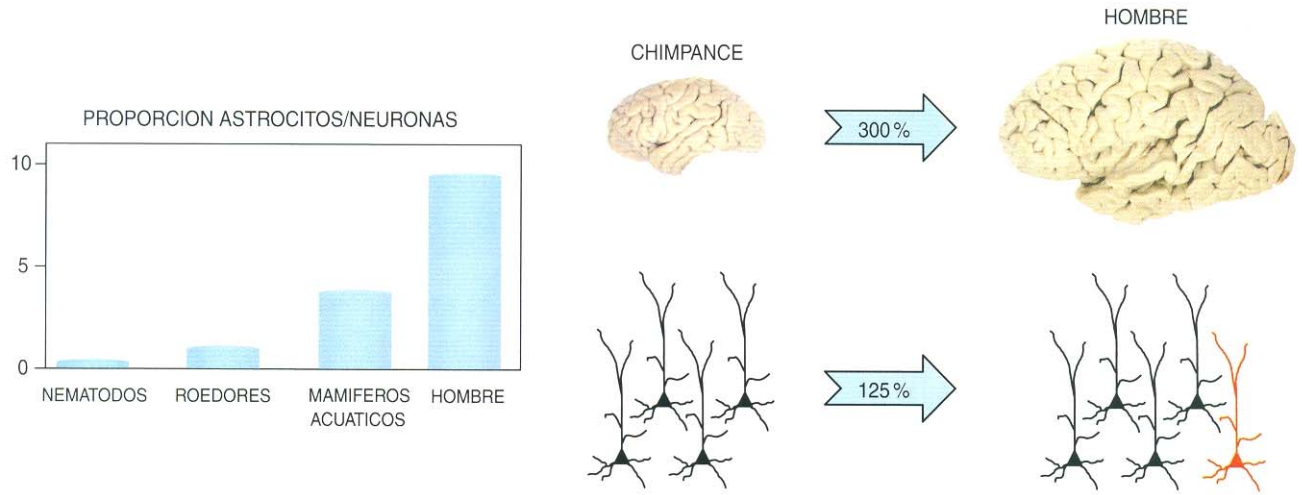
neurotransmisores. Los astrocitos del hipocampo, región del cerebro relacionada con procesos de aprendizaje y memoria, responden selectivamente con aumentos del calcio intracelular a la activación de distintas sinapsis que liberan acetilcolina y glutamato. La estimulación simultánea de ambos tipos de sinapsis pone de manifiesto la capacidad de los astrocitos de procesar e integrar la información sináptica.

Cuando se activan simultáneamente las sinapsis de acetilcolina y glutamato, la respuesta de los astrocitos no es la suma lineal (aditiva) de las respuestas observadas cuando una y otra sinapsis se activan por separado. Si se activan simultáneamente a unas frecuencias relativamente altas, se observa una depresión de la señal de calcio astrocitaria, mientras que a bajas frecuencias de estimulación la respuesta astrocitaria se ve aumentada. Con otras palabras, los astrocitos actúan como un control de ganancia, de manera que, cuando la actividad de dos sinapsis distintas es alta, su respuesta es reducida, mientras que cuando aquélla es baja, su respuesta resulta amplificada. Así pues, la capacidad de integración de la información por los astrocitos se manifiesta como una modulación no lineal de la señal de calcio en respuesta a la actividad simultánea de distintas sinapsis.

La modulación de la señal de calcio puede generarse por aplicación directa de los neurotransmisores glutamato y acetilcolina en ausencia de actividad neuronal. Significa ello que la modulación de la señal de calcio depende de propiedades celulares intrínsecas de los astrocitos.

El fenómeno de modulación de la señal de calcio tiene lugar en los microdominios, unas regiones concretas de los procesos astrocitarios; por ese motivo podemos considerarlos la unidad elemental en la comunicación neurona-astrocito. Además, la modulación de la señal de calcio controla la propagación intracelular del calcio a lo largo del astrocito.

Se desconocen los mecanismos que determinan si esta señal de calcio queda limitada localmente en cada microdominio o si se extiende a otras regiones adyacentes. Lo que no impide que encierren un significado funcional relevante, ya que determinan la extensión de la señal intracelular que provoca la liberación



6. INCREMENTO EN LA PROPORCION DE ASTROCITOS a lo largo de la escala evolutiva. El volumen cerebral de un hombre es un 300 % mayor que el de un chimpancé; sin embargo, su número de neuronas es sólo un 125 % mayor.

de gliotransmisores por el astrocito y la consiguiente expansión espacial de los fenómenos de modulación de la actividad sináptica.

Del análisis histológico se desprende que un astrocito de hipocampo de rata adulta ocupa un volumen medio de ≈66.000 mm^3, y por tanto puede interaccionar con ≈140.000 sinapsis. Dicho de otro modo: un solo astrocito puede actuar sobre un número alto de sinapsis, diferencialmente moduladas según la extensión de la señal de calcio intracelular; tamaña capacidad permite un sutil grado de comunicación entre astrocitos y neuronas.

Los astrocitos modulan la actividad neuronal

Conocida la existencia de excitabilidad en los astrocitos, era lógico preguntarse sobre las posibles consecuencias que las variaciones de calcio comportarían en la acción de las neuronas. La investigación reciente de varios laboratorios, incluido el nuestro, ha demostrado que no sólo las neuronas liberan neurotransmisores químicos, sino que los astrocitos pueden también liberar los gliotransmisores glutamato, ATP, D-serina, adenosina, GABA o TNF α, cuando aumentan sus niveles de calcio intracelular.

Se han identificado, además, los mecanismos celulares y moleculares responsables de la secreción de tales moléculas transmisoras. Se trata de mecanismos esencialmente idénticos a los de liberación de neurotransmisores por neuronas. Así, aunque en condiciones patológicas pueden existir mecanismos alternativos, la mayoría de los gliotransmisores son liberados por exocitosis vesicular regulada por los niveles de calcio intracelular.

Observamos, pues, que la señal de calcio en los astrocitos puede generar señales de retroalimentación hacia las neuronas. Vale decir: además de la comunicación descrita entre neuronas y astrocitos, éstos pueden comunicarse con las neuronas, estableciéndose así una comunicación bidireccional entre astrocitos y neuronas. Igual que la comunicación neurona-astrocito se basa en el control neuronal de la señal de calcio astrocitaria, resulta clave también dicha señal en los procesos de comunicación astrocito-neurona. A través de la liberación de gliotransmisores, la señal de calcio participa en la modulación de la excitabilidad neuronal y la transmisión sináptica.

La señalización entre astrocitos y neuronas puede manifestarse en las propiedades eléctricas de las neuronas. Nuestros estudios, iniciados en cultivos celulares de neuronas y astrocitos y confirmados recientemente en rodajas de cerebro de rata, han demostrado que el aumento del Ca^{2+} intracelular en los astrocitos genera, en las neuronas adyacentes, corrientes lentas de entrada (que bautizamos como SIC, del inglés *Slow Inward Current*), debidas a la liberación del gliotransmisor glutamato por los astrocitos y la consiguiente activación de receptores de glutamato en las neuronas. Estas SIC son responsables de variaciones del potencial de membrana neuronal. Por tanto, los astrocitos pueden modular la excitabilidad eléctrica de las neuronas.

Pero no sólo la excitabilidad neuronal se halla bajo el control de la actividad de los astrocitos. La propia transmisión sináptica es modulada por gliotransmisores liberados por los astrocitos. En estudios realizados, en un principio, sobre cultivos celulares demostramos que el glutamato liberado por los astrocitos podía modular transitoriamente las corrientes sinápticas, en otras palabras, los astrocitos podían regular la eficacia de la neurotransmisión, con la repercusión consiguiente en la transferencia de información entre neuronas.

Con la expresión "plasticidad sináptica" se alude a la capacidad del sistema nervioso para modificar de manera temporal o permanente la eficacia de la conexión sináptica entre las neuronas. Se producen fenómenos de plasticidad sináptica durante la maduración del sistema nervioso y después de la misma. Se les supone

responsables de los procesos celulares de memoria y aprendizaje.

La posible participación de los astrocitos en los procesos de plasticidad sináptica de larga duración es objeto de interés de numerosos laboratorios, incluido el nuestro. La investigación reciente sobre el hipotálamo de rata por el grupo de S. Oliet ha puesto de manifiesto que los cambios estructurales en la disposición física entre astrocitos y sinapsis que ocurren en distintos estados fisiológicos del animal (durante la lactancia) pueden regular la neurotransmisión en dicha región cerebral.

Los astrocitos del hipotálamo liberan el gliotransmisor D-serina, necesario para la neurotransmisión mediada por un tipo especial de receptor de glutamato denominado NMDA. En ratas que se encuentran en período de lactancia existe una retracción de los procesos astrocitarios y, por tanto, una reducción de la proximidad física entre astrocitos y sinapsis, lo que da lugar a una menor disponibilidad de D-serina por las sinapsis y, por tanto, a una menor activación de receptores de NMDA. En tales condiciones, queda mermada la plasticidad de las sinapsis, lo que sugiere que la capacidad de aprendizaje y memoria de las sinapsis depende de su relación espacial con los astrocitos adyacentes.

Más allá de esos cambios plásticos de la neurotransmisión basados en cambios estructurales que regulan pasivamente la plasticidad sináptica, nuestros resultados experimentales llevados a cabo en rodajas de hipocampo de rata revelan que el aumento de calcio en astrocitos de hipocampo puede dar lugar a una modulación persistente de larga duración de la transmisión sináptica. Se daría, pues, una participación activa de los astrocitos en la generación de plasticidad sináptica.

La comunicación entre neuronas ocurre en milisegundos; la velocidad de propagación de la información por potenciales de acción a lo largo de los procesos neuronales es del orden de metros por segundo. En cambio, los procesos de excitabilidad y comunicación en que intervienen los astrocitos se desarrollan en escalas de tiempo y velocidades varios órdenes de magnitud superiores y más lentos (decenas de segundos y micras por segundo, respectivamente).

Sin duda, los mecanismos rápidos de transferencia de información tienen notables ventajas adaptativas y resultan esenciales en el reino animal, pero cabe conjeturar que los procesos lentos moduladores, como los descritos sobre los astrocitos, pueden ser idóneos para un exquisito ajuste y refinamiento en el procesamiento complejo de información y en los procesos de plasticidad; en definitiva, en las funciones superiores del SNC. Expresado llanamente, para huir de un león es necesaria la rápida conducción de información desde el sistema visual al sistema motor, mas para idear una trampa que nos permita cazar un león no se requiere rapidez, sino una gran capacidad de modulación de información. Quizás ahí resida la importancia de la comunicación astrocito-neurona.

Con todo, habrá que buscar pruebas que relacionen los procesos cognitivos con la actividad astrocitaria. Sabido es que, a lo largo de la escala filogenética, se multiplica el número de neuronas. Crece también la proporción de células gliales. Así, la proporción de células gliales respecto al número de neuronas es inferior a uno en nematodos, uno en roedores, cuatro en mamíferos acuáticos y alrededor de diez en primates. La mayor cantidad relativa de astrocitos se da en el cerebro humano; aquí la población de astrocitos decuplica la de neuronas.

Además, resulta una idea atrayente constatar que el volumen del cerebro humano es un 300 % mayor que el de los otros primates; en cambio, su número de neuronas es sólo un 125 % mayor. Por tanto, la gran diferencia existente entre el volumen cerebral entre humanos y primates se debe no sólo a un incremento en el desarrollo del neurópilo neuronal, sino también al aumento del número y la complejidad de los astrocitos. Como dejó dicho Cajal en 1913,

> *"la corteza cerebral humana discrepa de la de los animales no sólo por la cantidad enorme de células de tipo glandular* [astrocitos] *que contiene, sino por la pequeñez de éstas* [y] *la riqueza del plexo gliomatoso intersticial"*.

En conclusión, la idea clásica de las células gliales en general y los astrocitos en particular como meras células de soporte trófico y estructural, sin función alguna en el procesamiento de información del sistema nervioso, ha quedado desmentida por las pruebas experimentales obtenidas en los últimos años. De acuerdo con las mismas, los astrocitos son elementos activos del procesamiento, transferencia y almacenamiento de información por el sistema nervioso.

Cien años después del trabajo original de Cajal sobre la significación fisiológica de la neuroglía, empezamos a reconocer el importante papel de los astrocitos en la fisiología neuronal y a vislumbrar la verdadera dimensión de la intervención de estas células en el funcionamiento del sistema nervioso.

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Review

Cell PRESS

Tripartite synapses: astrocytes process and control synaptic information

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The term 'tripartite synapse' refers to a concept in synaptic physiology based on the demonstration of the existence of bidirectional communication between astrocytes and neurons. Consistent with this concept, in addition to the classic 'bipartite' information flow between the pre- and postsynaptic neurons, astrocytes exchange information with the synaptic neuronal elements, responding to synaptic activity and, in turn, regulating synaptic transmission. Because recent evidence has demonstrated that astrocytes integrate and process synaptic information and control synaptic transmission and plasticity, astrocytes, being active partners in synaptic function, are cellular elements involved in the processing, transfer and storage of information by the nervous system. Consequently, in contrast to the classically accepted paradigm that brain function results exclusively from neuronal activity, there is an emerging view, which we review herein, in which brain function actually arises from the coordinated activity of a network comprising both neurons and glia.

Introduction

Ten years ago the term 'tripartite synapse' was proposed to conceptualize the evidence obtained by many laboratories during the 1990s that revealed the existence of bidirectional communication between neurons and astrocytes (Figure 1). It represents a new concept in synaptic physiology wherein, in addition to the information flow between the pre- and postsynaptic neurons, astrocytes exchange information with the synaptic neuronal elements, responding to synaptic activity and regulating synaptic transmission [1] (Figure 2). The biology of astrocyte–neuron interaction has emerged as a rapidly expanding field and has become one of the most exciting topics in current neuroscience that is changing our vision of the physiology of the nervous system. The classically accepted paradigm that brain function results exclusively from neuronal activity is being challenged by accumulating evidence suggesting that brain function might actually arise from the concerted activity of a neuron–glia network.

Here, we briefly summarize early evidence that led to the establishment of the concept of a tripartite synapse and then discuss more recent data regarding the properties and physiological consequences of the astrocyte Ca^{2+} signal, which has a fundamental role in neuron–astrocyte communication as the cellular signal triggered by the neuronal activity and responsible for transmitter release from astrocytes and the consequent neuromodulation. Although astrocytes have important roles in key aspects of brain development and function, such as neuronal metabolism, synaptogenesis, homeostasis of the extracellular milieu, or cerebral microcirculation [2], we focus on the role of astrocytes in synaptic physiology, discussing data indicating that astrocytes integrate and process synaptic information and finally regulate synaptic transmission and plasticity through the release of gliotransmitters (i.e. transmitters released by glial cells implicated in rapid glial–neuron and glial–glial communication) [3].

Ca^{2+}-mediated cellular excitability of astrocytes

The astrocytic revolution in current neuroscience began in the early 1990s when pioneering studies used the fluorescence imaging techniques to monitor intracellular Ca^{2+} levels in living astrocytes. Those studies revealed that cultured astrocytes display a form of excitability based on variations of the intracellular Ca^{2+} concentration [4,5]. Until then, astrocytes had been considered as non-excitable cells because, unlike neurons, they do not show electrical excitability (e.g. see Refs [6–9]). Since these pioneering findings, subsequent studies performed in cultured cells, brain slices and, more recently, *in vivo* have firmly established the astrocyte excitability, which is manifested as elevations of cytosolic Ca^{2+} mainly as a result of the mobilization of Ca^{2+} stored in the endoplasmic reticulum. The elevated Ca^{2+} then acts as a cellular signal [10]. Whereas neurons base their cellular excitability on electrical signals generated across the plasma membrane [11], astrocytes base their cellular excitability on variations of Ca^{2+} concentration in the cytoplasm.

Astrocyte Ca^{2+} signal is controlled by synaptic activity

Astrocyte Ca^{2+} elevations can occur spontaneously as intrinsic oscillations in the absence of neuronal activity [12–15], and they can also be triggered by neurotransmitters released during synaptic activity [10] (Table 1), which is of crucial importance because it indicates the existence of neuron-to-astrocyte communication (Figure 3a).

The synaptic control of the astrocyte Ca^{2+} signal is based on the fact that astrocytes express a wide variety of functional neurotransmitter receptors. Many of these receptors are of metabotropic type, being associated with G proteins that, upon activation, stimulate phospholipase C and formation of inositol (1,4,5)-triphosphate (Ins(1,4,5)P_3), which increases the intracellular Ca^{2+} concentration through the release of Ca^{2+} from intracellular Ins(1,4,5)P_3-sensitive Ca^{2+} stores [16–21]. Early studies using cultured cells showed that the astrocyte Ca^{2+} signal can propagate to

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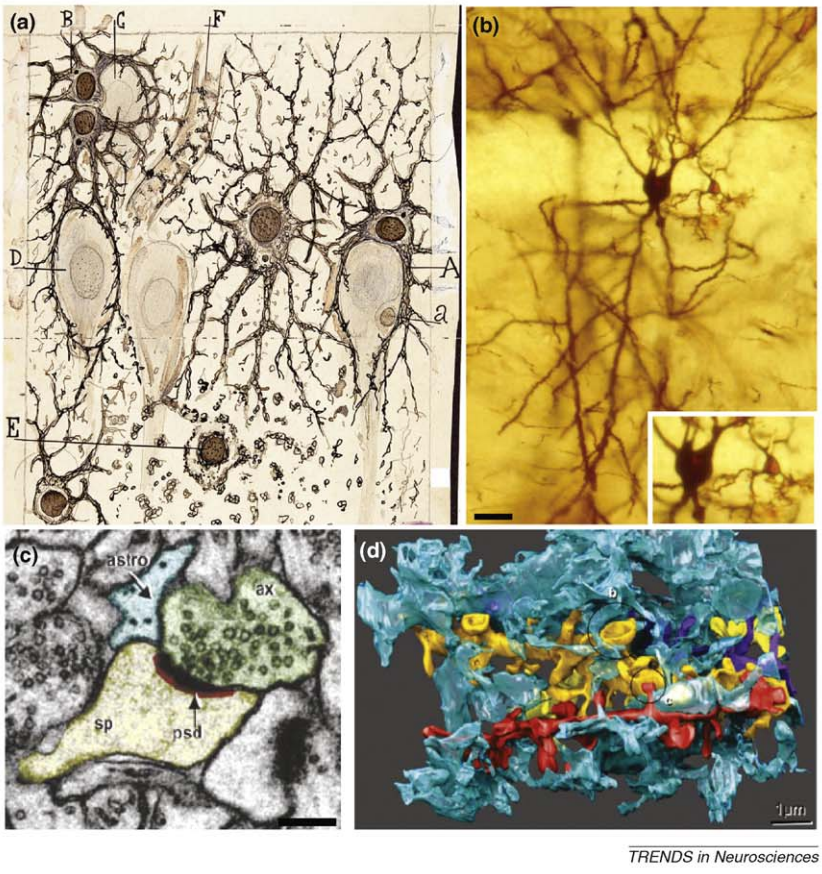

Figure 1. Views of the neuron–astrocyte interaction at the tripartite synapse. **(a)** Cajal's drawing showing 'neuroglia' of the pyramidal layer and stratum radiatum of the Ammon horn (from adult man autopsied three hours after death). Original labels: A, large astrocyte embracing a pyramidal neuron; B, twin astrocytes forming a nest around a cell, C, while one of them sends two branches forming another nest, D; E, cell with signs of 'autolysis'; F, capillary vessel. Reproduced from an original drawing, with permission of the Instituto Cajal [106]. **(b)** Neuron and astrocyte stained with the Golgi method from a rat hippocampus. Inset: astrocyte and neuronal somas. Image generously given by Dr Lopez-Mascaraque (Instituto Cajal). **(c)** Electron microscopy image of astrocyte process at the axon–spine interface: astrocyte process (astro, blue); postsynaptic density (psd, red); dendritic spine head (sp, yellow); axonal bouton (ax, green). Reproduced, with permission, from Ref. [107]. (d) 3D reconstruction of a single astrocyte process (blue) interdigitating among four dendrites (gold, yellow, red and purple). Reproduced, with permission, from Ref. [107].

neighboring astrocytes as an intercellular Ca^{2+} wave involving dozens of cells [4,5,22]. By contrast, in brain slices such waves seem to involve few astrocytes, and their actual existence in more intact preparations is currently under debate [23]. The synaptically evoked as well as the spontaneous Ca^{2+} signal originates in spatially restricted areas – called 'microdomains' – of the astrocyte processes [24,25] from where it can eventually propagate intracellularly to other regions of the cell [20,25,26]. As a single astrocyte might contact ~100 000 synapses [27], the control of the spatial extension of the Ca^{2+} signal could have relevant functional consequences for the physiology of the nervous system, because not all synapses covered by a single astrocyte are necessarily functionally locked to be similarly and simultaneously modulated (see below). Therefore, differential neuromodulation of specific synapses would provide an extraordinary increase of the degrees of freedom to the system [28,29].

Astrocyte Ca^{2+} signal *in vivo*

For many years, technical constraints limited astrocyte Ca^{2+}-signal studies to cultured cells and brain slices. The recent use of novel imaging techniques, that is, two-photon microscopy and specific fluorescent dyes that selectively label astrocytes *in vivo* [30], which enable the study of astrocyte Ca^{2+} signals in the whole animal, has revealed important findings (Figure 3b). First, reports from studies of rat, mouse and ferret have demonstrated that astrocytes *in vivo* exhibit intracellular Ca^{2+} variations, indicating that astrocyte Ca^{2+} excitability is not a peculiarity of slice preparations. Second, like in brain slices, astrocyte Ca^{2+} variations occur spontaneously [30–33] and are also evoked by neurotransmitters released during synaptic activity [31,33–37], indicating that neuron-to-astrocyte communication is present *in vivo*. Finally, and of special relevance, astrocyte Ca^{2+} elevations might be triggered by physiological sensory stimuli. Indeed, stimulation of whiskers increased the astrocyte Ca^{2+} in mouse barrel cortex [33] (Figure 3b). Astrocytes of the sensory cortex also elevate their Ca^{2+} in response to a robust peripheral stimulation that is known to activate the locus coeruleus or to direct electrical stimulation of this nucleus [34], as well as during running behavior in alert mice [35]. Astrocytes from other brain regions also respond to stimuli of corresponding sensory modalities. Astrocytes in the visual cortex not only show Ca^{2+} elevations in response to visual stimuli but also the properties of these responses indicate the existence of distinct spatial receptive fields and reveal an even sharper

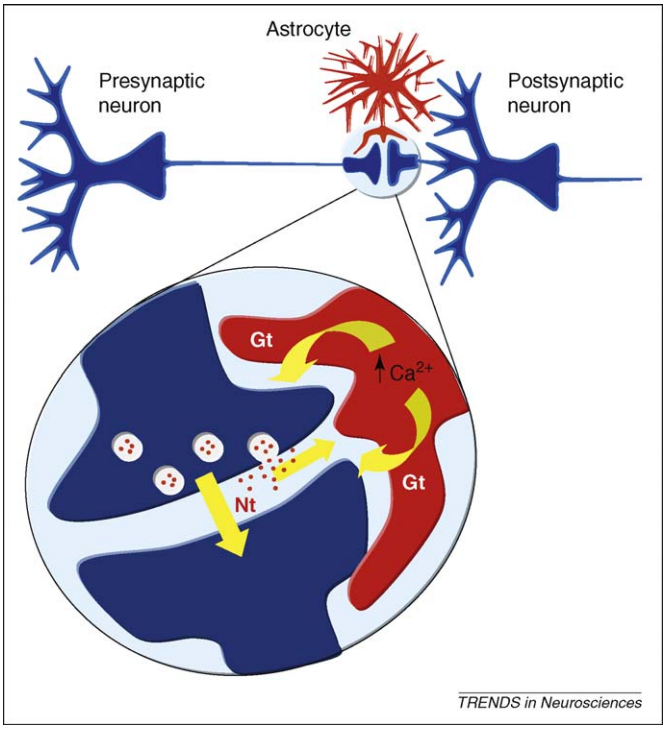

Figure 2. Scheme of the tripartite synapse. Cartoon representing the transfer of information between neuronal elements and astrocyte at the tripartite synapse. Astrocytes respond with Ca^{2+} elevations to neurotransmitters (Nt) released during synaptic activity and, in turn, control neuronal excitability and synaptic transmission through the Ca^{2+}-dependent release of gliotransmitters (Gt).

tuning than neurons to visual stimuli [37]. In summary, astrocytes *in vivo* display Ca^{2+} excitability and respond to neuronal activity. Furthermore, because astrocytes in specific sensory areas respond to a variety of sensory stimuli, it is feasible that astrocytes participate in the brain representation of the external world.

Synaptic information processing by astrocytes

In contrast to the view of astrocytes as passive elements that provide the adequate environmental conditions for appropriate neuronal function and that respond to neurotransmitters, simply performing a linear readout of the synaptic activity, experimental evidence supports the idea that astrocytes integrate and process synaptic information elaborating a complex nonlinear response to the incoming information from adjacent synapses (Box 1). As described earlier, it is firmly established that astrocytes respond with Ca^{2+} elevations to synaptic activity [25]. However, to understand the actual role of astrocytes in brain information processing, it is necessary to define whether the astrocyte Ca^{2+} signal passively results from different neurotransmitter concentrations attained during synaptic activity or, alternatively, whether neuron-to-astrocyte communication presents properties of complex information processing that are classically considered to be exclusive to neuron-to-neuron communication. In Box 1 and in the following discussion we will elaborate the evidence that supports the idea that astrocytes are cellular processors of synaptic information.

Astrocytes discriminate the activity of different synaptic pathways

The astrocyte Ca^{2+} signal does not result from a nonspecific spillover of neurotransmitters; instead, it is selectively mediated by the activity of specific synaptic terminals (Figure 4). Astrocytes located in the *stratum oriens* of the CA1 area of the hippocampus respond to the stimulation of the alveus (which contains glutamatergic and cholinergic axons) with Ca^{2+} elevations that are specifically mediated by acetylcholine (ACh) but not by glutamate [16]. By contrast, these astrocytes do respond to glutamate when it is released by different glutamatergic synapses, that is, the Schaffer collateral (SC) synaptic terminals [25]. Hence, astrocytes selectively respond to different synapses that use different neurotransmitters (i.e. glutamate and ACh), and they discriminate between the activity of different pathways that use the same neurotransmitter (i.e. glutamatergic axons of SC and alveus) [25]. Likewise, astrocytes in the ventrobasal thalamus respond to the

Table 1. Ca^{2+} signaling in astrocytes

	Neurotransmitter	Experimental model	Brain area	Refs
Spontaneous activity	Non-applicable	Brain slices	Thalamus	[12,14]
			Hippocampus	[12,13]
			Cerebellum	[12,24]
			Cortex	[15]
			Striatum	[12]
		In vivo	Cortex	[31,32,34–36]
Synaptically evoked	Norepinephrine	Brain slices	Cerebellum	[19]
		In vivo	Cortex	[34]
	ATP	Brain slices	Hippocampus	[81]
			Cerebellum	[82,83]
			Retina	[84]
			Olfatory bulb	[85]
	GABA	Brain slices	Hippocampus	[18,70]
	Glutamate	Brain slices	Hippocampus	[16,17,20,25,86]
			Cortex	[20,39]
			Nucleus accumbens	[61]
			Cerebellum	[82,83]
			Olfatory Bulb	[85]
		In vivo	Cortex	[33,37]
	Acetylcholine	Brain slices	Hippocampus	[16,25]
	Nitric Oxide	Brain slices	Cerebellum	[87]
	Endocannabinoids	Brain slices	Hippocampus	[64]

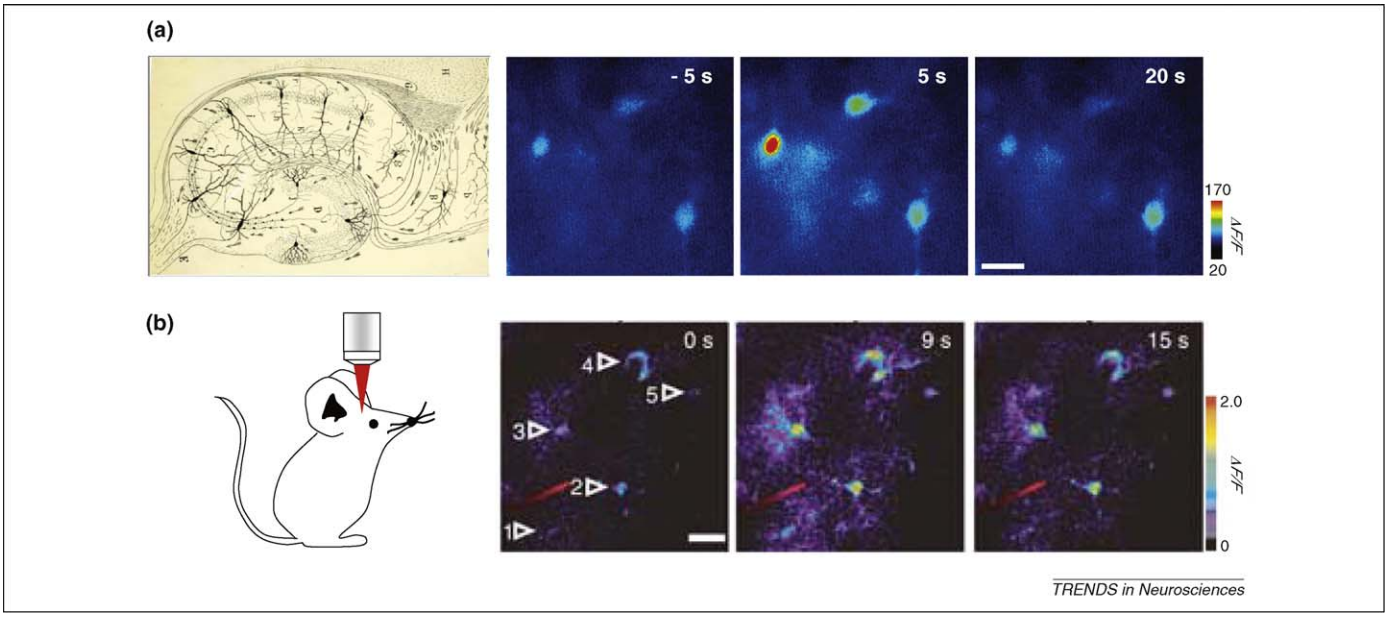

Figure 3. Astrocyte Ca^{2+} signaling in brain slices and *in vivo*. (a) Cajal's drawing of the mammalian hippocampus (reproduced from an original drawing with permission of the Instituto Cajal) and pseudocolor images from rat hippocampal slices representing fluorescence intensities indicative of astrocyte Ca^{2+} levels before (−5 s) and after (5 s, 20 s) electrical stimulation of Schaffer collaterals. Scale bar, 10 μm. (b) Two-photon microscopy images of the *in vivo* astrocyte Ca^{2+} signal in the barrel cortex. Pseudocolor images represent fluorescence intensities indicative of astrocyte Ca^{2+} levels before (0 s) and after (9 s, 15 s) evoked by whisker stimulation. Scale bar, 20 μm. Reproduced, with permission, from Ref. [33]. Note the astrocyte Ca^{2+} elevations evoked by electrical synaptic and sensory stimulation in hippocampal slices (a) and *in vivo* barrel cortex (b), respectively.

stimulation of either sensory or corticothalamic pathways, but very few respond to the activity of both [38]. Furthermore, astrocytes in the barrel cortex also respond selectively to the activity of different neuronal inputs, because astrocytes in layer 2/3 respond to glutamatergic inputs from layer 4 in the same column but not to glutamatergic projections from layer 2/3 of adjacent columns [39] (Figure 4b). Therefore, astrocytes show selective responses that discriminate the activity of specific synapses.

Astrocyte Ca^{2+} signals show a nonlinear relationship with the synaptic activity

The analysis of the astrocyte Ca^{2+} signal evoked by the activity of different synaptic terminals that release ACh and glutamate indicates that astrocytes integrate synaptic information [25]. In hippocampal slices, the simultaneous stimulation of alveus and SC (that elicit Ca^{2+} elevations mediated by ACh and glutamate, respectively) evokes astrocytic responses that are inconsistent with a linear readout of the synaptic activity. The amplitude of the Ca^{2+} elevations elicited by simultaneous stimulation of both pathways is not equivalent to the linear summation of the Ca^{2+} signals evoked by independent stimulation [25] (Figure 4a). Therefore, the astrocyte Ca^{2+} signal is nonlinearly modulated by the simultaneous activity of cholinergic and glutamatergic synapses. Moreover, while the Ca^{2+} signal evoked by simultaneous stimulation at high frequencies (30 and 50 Hz) displays a sublinear summation of the responses evoked independently, it shows a supralinear summation after stimulation at relatively low frequencies (1 and 10 Hz); that is, the Ca^{2+} signal is relatively depressed or potentiated at relative high and low frequencies of neuronal activity, respectively. Therefore, the astrocyte Ca^{2+} signal is nonlinearly modulated by the simultaneous activity of different synaptic inputs, and the sign of this modulation depends on the synaptic activity level [25].

Astrocytes have cell-intrinsic properties

The astrocyte Ca^{2+} signal evoked by exogenously applied neurotransmitters can have synergistic effects [40–42]. Furthermore, the modulation of the Ca^{2+} signal in hippocampal astrocytes described earlier occurs in the absence of synaptic activity when the transmitters glutamate and ACh are applied [25]. Interestingly, the Ca^{2+} signal evoked by the simultaneous application of glutamate and γ-aminobutyric acid (GABA) is equal to the linear summation of the Ca^{2+} elevations evoked independently, indicating that the astrocyte Ca^{2+} signal modulation depends on the transmitters involved, probably owing to the activation of different intracellular signaling cascades. Indeed, the intracellular signaling pathways of both metabotropic ACh and glutamate receptors converge at the activation of the phospholipase C, whereas $GABA_B$ receptors are coupled to different intracellular pathways that involve adenylate cyclase regulation [29]. Hence, the astrocyte Ca^{2+}-signal modulation is a specific phenomenon that depends on the neurotransmitters involved and, consequently, might be selectively induced by specific synaptic pathways. Therefore, astrocytes are endowed with cell-intrinsic properties that grant the nonlinear responses to the synaptic activity and that are probably determined by the intracellular signaling events, like intrinsic properties of neurons are based on the electrical properties of the membrane.

In summary, these findings indicate that astrocytes are cellular elements involved in the information processing by the nervous system. Although our current knowledge of the

Box 1. Astrocytes integrate and process synaptic information

One of the most relevant functional properties of neurons, which are responsible for their role in the brain information processing, is based on the fact that neuronal electrical excitability is nonlinearly regulated by the simultaneous activity of different converging synaptic inputs. Neurons receive thousands of input signals in the form of synaptic potentials that are integrated nonlinearly in the soma and dendrites to elaborate a single output signal in form of action potentials. This nonlinear integration of the multiple incoming input signals is considered to represent the fundamental basis of the information processing by neurons and is the heart of the nervous system activity. Neuronal information processing is based on two key functional properties of neurons: (i) selective responsiveness to different specific synaptic inputs, and (ii) neuronal intrinsic properties determined by the expression of a plethora of voltage- and ligand-gated channels and membrane electrical properties. These properties account for the complex nonlinear input–output relationships that are responsible for the integrative properties of neurons [108,109].

The demonstration that astrocytes are excitable cells that base their excitability on variations of the intracellular Ca^{2+} signal that can be triggered by neurotransmitters released during synaptic activity [25] raises the question of whether astrocytes integrate and process synaptic information, challenging the classical idea that synaptic information processing is exclusive to neurons.

Two possible views arise from the synaptic regulation of the astrocyte Ca^{2+} signal. (i) While the duration, amplitude and frequency of the astrocyte Ca^{2+} signal are regulated by different levels of synaptic activity [29], the different responses might passively result from different neurotransmitter concentrations attained during different levels of synaptic activity. Consequently, astrocytes would perform a linear readout of the synaptic activity, where the astrocyte Ca^{2+} signal would simply reflect the synaptic activity level. (ii) Alternatively, astrocytes might integrate and process synaptic information, elaborating a complex nonlinear response to the incoming input signals received from adjacent synapses.

To distinguish between both alternative views, we propose the following simplest criteria that astrocytes must meet to be considered as cellular processors of synaptic information:

(i) To have cellular excitability.
(ii) To show selective responsiveness to specific synaptic inputs.
(iii) To display nonlinear input–output relationships.
(iv) To have cell-intrinsic properties.

As detailed in the text, several pieces of evidence indicate that astrocytes satisfy these requirements:

(i) the astrocyte cellular excitability based on intracellular calcium variations has been firmly established in culture, slices and *in vivo* preparations; this Ca^{2+} excitability might be present as spontaneous intrinsic oscillations [12–14] and might be triggered by neurotransmitters released from synaptic terminals [16,18,20,24,86] as well as from postsynaptic neurons [64].
(ii) Hippocampal astrocytes selectively respond to different synapses that use different neurotransmitters [25]. Furthermore, astrocytes in the hippocampus [25], ventrobasal thalamus [38] and barrel cortex [39] can discriminate between the activity of different synaptic pathways that use glutamate as neurotransmitter, selectively responding to specific neuronal pathways. Therefore, astrocytes show selective responses that discriminate the activity of specific synapses.
(iii) The amplitude of the astrocyte Ca^{2+} signal is nonlinearly modulated by the simultaneous activity of different synaptic pathways that use glutamate and acetylcholine as neurotransmitters, showing sublinear or supralinear summation at relatively high or low levels of synaptic activity, respectively [25]. Hence, astrocytes accommodate nonlinearly their Ca^{2+} signal to the different simultaneously active synapses and to their activity level.
(iv) The astrocyte Ca^{2+} signal is nonlinearly modulated by simultaneous exogenous application of different neurotransmitters. Therefore, astrocytes are endowed with cell-intrinsic properties that grant the nonlinear responsiveness to the synaptic activity. These cell-intrinsic properties of astrocytes probably reside in the intracellular signaling events, just like the intrinsic properties of neurons are determined by the electrical properties of their membranes.

These data indicate that astrocytes fulfill the requirements proposed and that, in addition to neurons, astrocytes too are cellular processors of information. Indeed, the properties of the astrocyte Ca^{2+} signal reveal that astrocytes integrate and process synaptic information, indicating that neuron-to-astrocyte communication presents attributes that were classically considered to be exclusive to neuron-to-neuron communication. Consequently, astrocytes are cellular elements involved in the information processing by the nervous system.

ability of astrocytes to process synaptic information has been gained from analysis performed in brain slices, *in vivo* studies are still required to appreciate the actual extent and importance of these properties on brain function.

Gliotransmission and modulation of synaptic transmission

One of the most stimulating topics in current neuroscience is the functional consequences of the astrocyte Ca^{2+} signal on neuronal physiology. Evidence obtained during the past 15 years has demonstrated that signaling between neurons and astrocytes is a reciprocal communication, where astrocytes not only respond to neuronal activity but also actively regulate neuronal and synaptic activity. Therefore, according to the concept of the tripartite synapse, to fully understand synaptic function, astrocytes must be considered as integral components of synapses where they have crucial roles in synaptic physiology.

Astrocytes release several neuroactive molecules, such as glutamate, D-serine, ATP, adenosine, GABA, tumor necrosis factor α (TNFα), prostaglandins, proteins and peptides, that can influence neuronal and synaptic physiology [3]. The mechanisms and consequences of this process, called gliotransmission, have attracted considerable interest. Several mechanisms of transmitter release from astrocytes have been proposed. Compelling evidence demonstrates that some transmitters are released in a Ca^{2+}-dependent manner [10,43–48] through vesicle [47–51] and lysosome [52–54] exocytosis. Furthermore, ultrastructural studies have shown that astrocytic processes contain small synaptic-like vesicles, which are located in close proximity to synapses, apposed either to presynaptic and postsynaptic elements [49,50]. Alternative release mechanisms, including reversal of glutamate transporters, connexin/pannexin hemichannels, pore-forming P2X7 receptors and swelling-induced activation of volume-regulated anion channels, have also been proposed (for a review, see Ref. [55]). Whether Ca^{2+}-dependent and -independent mechanisms coexist and under what physiological or pathological conditions they occur remain unclear.

The original demonstration of astrocyte-induced neuromodulation in cultured cells [43,44,56] has been considerably expanded by later studies on acute brain slices (for reviews, see Refs [57–59]; Table 2). Glutamate was one of the first gliotransmitters released from astrocytes to be identified and has been reported to exert many effects on

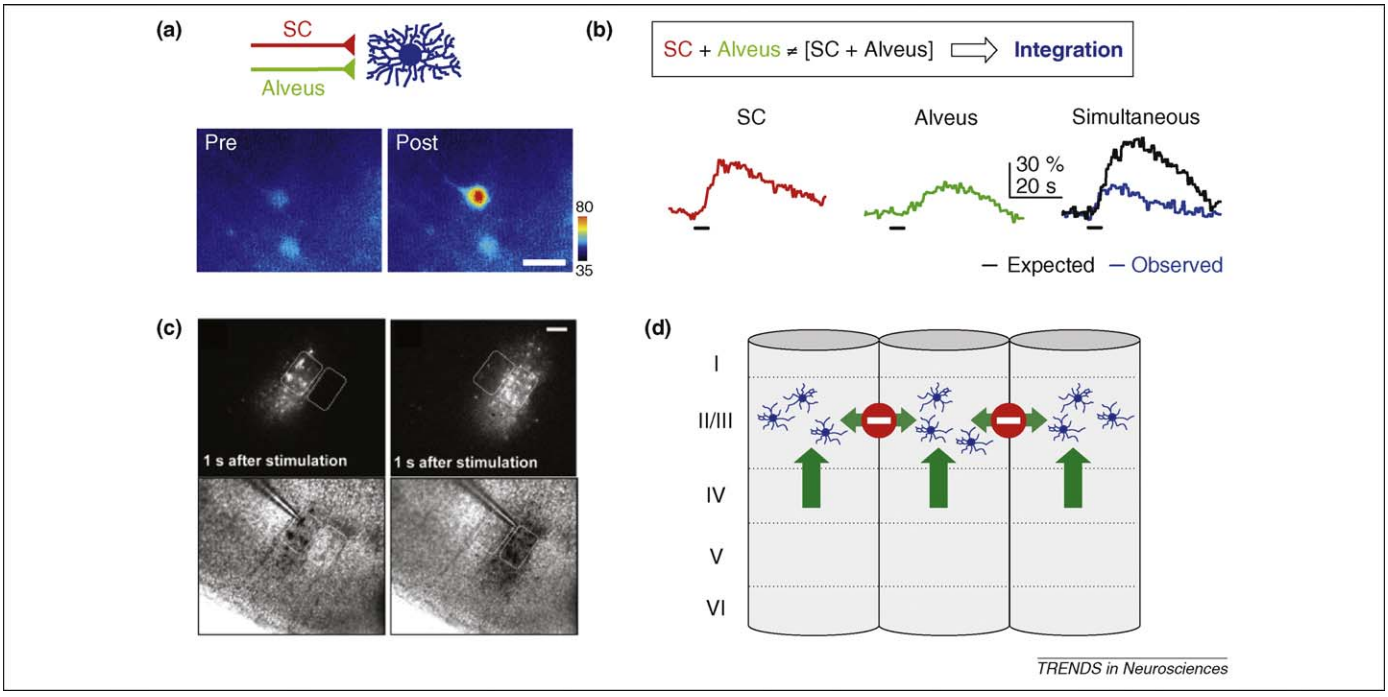

Figure 4. Astrocytes integrate synaptic information. **(a)** Schematic drawing and pseudocolor images of astrocyte Ca^{2+} elevations evoked by stimulation of Schaffer collaterals (SC, red) or alveus (green). Astrocytes integrate synaptic information from different synaptic inputs. Scale bar, 15 μm. **(b)** Hypothesis of astrocyte integration of synaptic information induced by SC and alveus activity (top) and astrocyte Ca^{2+} signals evoked by independent and simultaneous stimulation of SC and alveus (bottom). Blue and black traces correspond to the observed and expected responses (i.e. the linear summation of the responses evoked by independent stimulation of both pathways), respectively. Horizontal lines at the bottom of each trace represent the stimuli. Modified from Ref. [25]. Note the lack of correspondence between observed and expected responses, that is, the relative reduction of the observed response versus the linear summation of the responses evoked independently, which is indicative of synaptic integration. **(c)** Top, fluorescence images showing astrocytic Ca^{2+} signals evoked after electrical stimulation (responding cells are displayed in white) of two contiguous barrel cortex. Bottom, images showing an overlay of the bright-field image with the location of the stimulating pipette and the responding astrocytes (shown in black). The barrels in layer 4 are outlined by dotted white lines. Scale bar, 100 μm. Note that astrocytes that respond to the stimulation of the barrel column are located within the stimulated barrel column, and no astrocytes respond to the stimulation of the adjacent barrel column, which indicates the selectivity of astrocyte responses. Reproduced, with permission, from Ref. [39]. **(d)** Schematic drawing illustrating the discrimination and response selectivity of barrel cortex astrocytes to neuronal activity from layer IV but not from layer II/III of neighboring barrels.

neuronal excitability. Astrocytic glutamate evokes slow inward currents (SICs) through activation of postsynaptic *N*-methyl-D-aspartate (NMDA) receptors [25,44,60–65] and synchronously excites clusters of hippocampal pyramidal neurons, indicating that gliotransmission increases neuronal excitability and operates as a nonsynaptic mechanism for neuronal synchronization [60,62]. By contrast, astrocytic glutamate might also activate receptors localized at presynaptic terminals. Through activation of group I metabotropic glutamate receptors (mGluRs) [46,26] or NMDA receptors [50], astrocytes enhance the frequency of spontaneous and evoked excitatory synaptic currents. Alternatively, astrocytes induce the potentiation [20] or depression of inhibitory synaptic transmission by activation of presynaptic kainate [66] or II/III mGlu [67] receptors, respectively. Therefore, a single gliotransmitter can exert multiple effects depending on the sites of action and the activated receptor subtypes, which provides a high degree of complexity to astrocyte–neuron communication. This complexity becomes even higher when considering that other gliotransmitters, such as GABA, ATP, adenosine (a metabolic product of ATP) or D-serine, could act on the same neuron or act on different cell types, thus evoking distinctive responses [63,68–72]. Moreover, in hippocampal astrocytes, Ca^{2+} elevations induced by activation of PAR-1 receptors, but not $P2Y_1$ receptors, evoke NMDA-receptor-mediated SICs in pyramidal neurons [65], indicating that the Ca^{2+} signal evoked by activation of different receptors might not be equally competent to stimulate gliotransmitter release. A great effort has been made so far to identify different gliotransmitters and their potential modulatory actions, but it remains unknown whether different gliotransmitters are co-released or whether different gliotransmitters are released by different astrocytes or by different astrocytic processes or domains. It is also crucial to elucidate the specific incoming inputs, the molecular mechanisms and the physiological conditions that govern the precise release of each gliotransmitter. Intracellular regulatory mechanisms of release and spatially defined specific intercellular signaling pathways seem to be present to grant a coherent astrocyte–neuron communication (see later).

Besides glutamate, ATP and its product adenosine of astrocytic origin also control synaptic transmission [68–71]. Indeed, heterosynaptic depression of hippocampal synaptic transmission requires astrocyte release of ATP/adenosine [69–71], which is stimulated by the $GABA_B$-mediated astrocyte Ca^{2+} signal elicited by interneuron activity evoked by SC [70]. This represents a paradigmatic example of the consequences of coordinated neuron–glia network on synaptic function. Furthermore, it also shows that synaptically evoked astrocytic ATP might signal to other synapses, thus spreading neuronal information beyond activated synapses [70]. Likewise, glutamate from

Table 2. Gliotransmitters and synaptic transmission

Gliotransmitter	Experimental preparation	Neuromodulation	Refs
Glutamate	Hippocampus	Depression of evoked EPSCs and IPSCs	[43,67]
		Frequency increase of miniature PSCs	[44]
		Frequency increase of miniature IPSCs	[18]
		Frequency increase of spontaneous EPSCs	[50,26]
		Frequency increase of spontaneous IPSCs	[66]
		Postsynaptic SIC	[25,43,60,62,64,65,88–94]
		Increase of neuronal excitability	[17]
		Heterosynaptic depression	[95]
	Cortex	Postsynaptic SIC	[96]
	Ventro basal thalamus	Postsynaptic SIC	[14]
	Nucleus accumbens	Postsynaptic SIC	[61]
	Olfactory Bulb	Postsynaptic SIC	[63]
	Retina	Light-evoked neuronal activity	[97]
ATP/Adenosine	Cerebellum	Depression of spontaneous EPSCs	[98]
	Hippocampus	Heterosynaptic depresion of EPSCs	[70,71]
		Modulation of LTP	[69]
		Synaptic depression	[69]
	Hypothalamic paraventricular nucleus	Insertion of AMPA receptors	[99]
	Retina	Depression of light-evoked EPSCs	[100]
D-Serine	Hippocampus	Modulation of LTP	[101]
	Hipothalamic supraoptic Nucleus	Modulation of LTP	[72]
	Retina	Potentiate NMDA receptor transmission	[102]
TNFα	Hippocampus	Insertion of AMPA receptors	[74]
		Increase of synaptic scaling	[76]
GABA	Olfactory bulb	Postsynaptic SOC	[63]
Undefined (glutamate and/or nitric oxide)	Neuromuscular junction	Synaptic depression	[103,104]
		Synaptic potentiation	[105]

Abbreviations: EPSCs, excitatory postsynaptic currents; IPSCs, inhibitory postsynaptic currents; LTP, long-term potentiation; PSCs, postsynaptic currents; SIC, slow inward current; SOC, slow outward current.

astrocytes stimulated by endocannabinoid released during neuronal activity could signal to adjacent unconnected neurons [64], suggesting that astrocytes serve as a bridge for nonsynaptic communication between neurons. In conclusion, astrocytes not only influence the active synapses through short-range signaling but they might also have long-range effects on distant synapses.

Hippocampal slices are a useful experimental model to study synaptic transmission, and consequently they have been also widely used to analyze the astrocyte effects on synaptic transmission. Although a comprehensive characterization of the phenomenon in different brain areas is still lacking, glia-mediated synaptic transmission modulation has also been documented in retina, supraoptic nucleus and cerebellum, as well as at the neuromuscular junction in the peripheral nervous system (for reviews see Refs [58,73]). Finally, the effects of the activity of single astrocytes on single synapses have been investigated recently in the hippocampus by performing paired recordings from pyramidal neurons and single astrocytes while stimulating SC single synapses, that is, by experimentally isolating the tripartite synapse [46]. Astrocyte Ca^{2+} elevations transiently increase the probability of neurotransmitter release from presynaptic terminals, thus enhancing the synaptic efficacy (Figure 5). This effect is mediated by Ca^{2+}- and SNARE protein-dependent release of glutamate from astrocytes, which activates group I metabotropic glutamate receptors at the presynaptic terminal [46].

Astrocytes and synaptic plasticity

Astrocytes operate at lower time scales than synaptic neurotransmission. Whereas fast neurotransmission occurs in milliseconds, astrocytic effects on neuronal physiology last seconds or tens of seconds. In addition, astrocyte regulation of synaptic transmission runs on different time scales, because astrocytes can control transiently the synaptic strength (during seconds), and they can also contribute to long-term synaptic plasticity. Several mechanisms underlying the astrocyte effects on long-term potentiation (LTP) have been described. Some studies indicate a passive or tonic mode of action, in which astrocytes tonically suppress or potentiate synaptic transmission [69,72,74,75]. Astrocytes through ATP/adenosine release control the strength of the basal hippocampal synaptic activity by tonic suppression of neurotransmission, which results in an increase in the dynamic range for LTP [69]. In the hypothalamic supraoptic nucleus, changes in the astrocytic coverage of synapses influence NMDA-receptor-mediated synaptic responses due to changes in the ambient levels of D-serine released by astrocytes [72].

By contrast, astrocytes participate in the generation of LTP through a phasic signaling process, in which the temporal coincidence of the astrocyte Ca^{2+} signal and the postsynaptic neuronal activity induces LTP through the activation of presynaptic type I mGluRs by Ca^{2+}-dependent glutamate release from astrocytes [46]. These findings have expanded our traditional vision of the Hebbian LTP (a paradigm of synaptic plasticity based on the coincident activity of pre and postsynaptic neuronal elements) to include astrocytes as new sources of cellular signals involved in synaptic plasticity.

Astrocytes and animal behavior

The elucidation of the actual impact of astrocyte Ca^{2+} signaling and gliotransmission on animal behavior

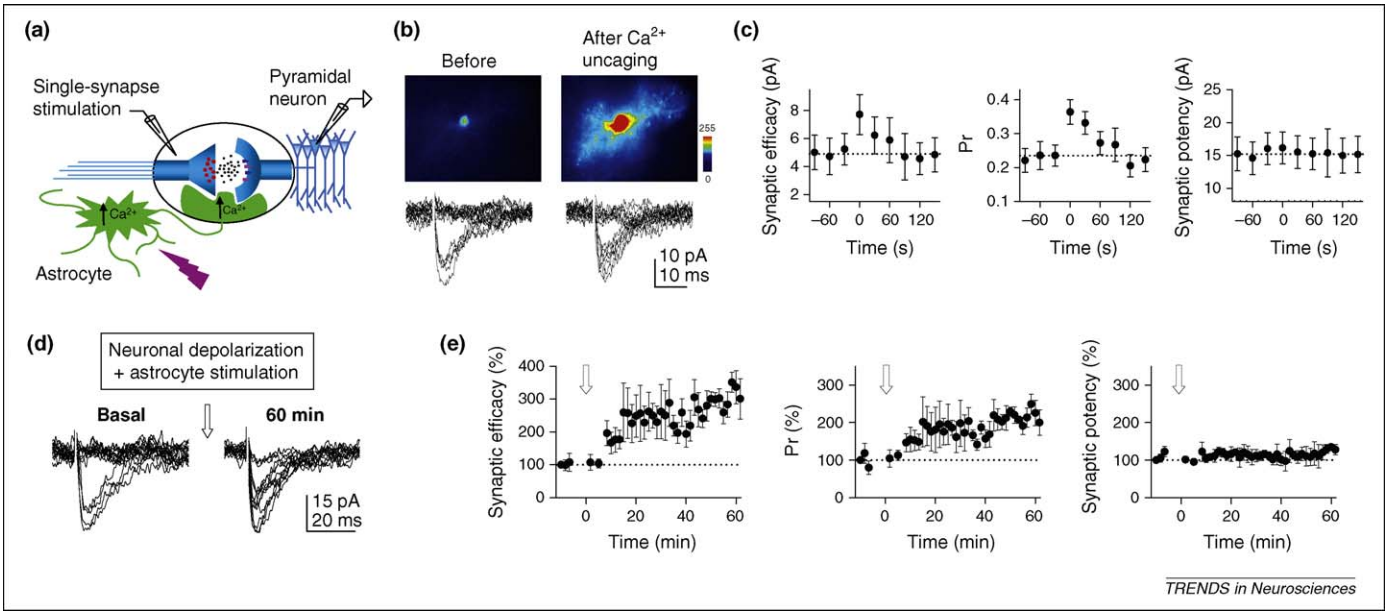

Figure 5. Astrocytes control synaptic transmission and plasticity at the tripartite synapse. **(a)** Schematic drawing showing recordings from one pyramidal neuron and one astrocyte, and the stimulation of a single synapse. **(b)** Astrocyte Ca^{2+} levels (top) and synaptic responses (bottom) before and after Ca^{2+} uncaging at a single astrocyte. Note the increase in the proportion of successful synaptic responses after astrocyte Ca^{2+} elevation. **(c)** Astrocytes potentiate synaptic efficacy (i.e. mean amplitude of all responses including failures), increasing the probability of transmitter release (Pr, ratio between the number of successes versus the total number of stimuli) without modulating the amplitude of synaptic responses (i.e. synaptic potency, defined as mean amplitude of the successful responses). Note the transient increase of synaptic efficacy and Pr after elevating astrocyte Ca^{2+} (at time zero). **(d,e)** Temporal coincidence of astrocyte Ca^{2+} signal and postsynaptic neuronal depolarization induces long-term potentiation (LTP) of synaptic transmission. Excitatory synaptic currents before and 60 min after transiently pairing neuronal depolarization and astrocyte Ca^{2+} uncaging. (e) Graphs showing relative changes in synaptic efficacy, Pr and synaptic potency parameters over time. Arrows indicate pairing of astrocyte Ca^{2+} signal and mild neuronal depolarization. Note the persistent potentiation of synaptic efficacy and Pr induced by the transient pairing (for 5 min) of neuronal and astrocyte stimulation (at time zero). Reproduced, with permission, from Ref. [46].

represents the ultimate challenge for the concept of the tripartite synapse. The development of transgenic animal models will be useful for this purpose. However, controversial data on this issue have been reported recently using different transgenic mice. Changes in hippocampal neuronal excitability and synaptic transmission were not detected when astrocyte Ca^{2+} elevations were evoked by selective activation of Mas-related G-protein-coupled receptor member A1 (MrgA1) receptors, a type of receptor coupled to Ca^{2+} release from internal stores that are not expressed endogenously in the brain but were transgenically expressed specifically in astrocytes of a transgenic mice [76]. Inherent problems of transgenic mice derived from the transgene expression under heterologous promoters, such as proper spatiotemporal expression of exogenous receptors as well as appropriate coupling to intracellular signaling cascades and cellular events (such as gliotransmitter release), might account for these negative results (for a discussion, see Ref. [23]). By contrast, a ground-breaking study has recently demonstrated that astrocytes contribute to the control of sleep homeostasis by using transgenic mice in which SNARE-dependent release of gliotransmitters from astrocytes was abolished. This study shows that adenosine metabolized from ATP released by astrocytes participates in the accumulation of sleep pressure and contributes to cognitive deficits associated with sleep loss [77]. Although they are not perfect experimental models, transgenic mice have the potential to reveal currently unknown roles for astrocytes in different brain functions.

Are all synapses tripartite?

Experiments designed to observe the effects of the astrocyte Ca^{2+} signal on single hippocampal synapses showed that not all recorded synapses displayed modulation of the synaptic efficacy after astrocyte stimulation, but only a subset of synapses (around 40%) underwent astrocyte-induced potentiation [46]. Experimental conditions might account for some ineffective cases because, owing to the limits of optical resolution, it could not be excluded that the stimulated astrocyte was not in sufficient close proximity to the recorded synapse. Alternatively, it is feasible that, in some cases, the stimulated astrocyte and the recorded synapse were not functionally connected. Whether this absence of connectivity is due to functional or structural bases is unknown, but it is interesting to note that ultrastructural data shows that only a subset of hippocampal excitatory synapses (again around 40%) are covered by astrocytic processes [78], which is consistent with the hypothesis that not all synapses are functionally tripartite. The fact that Ca^{2+} elevations evoked in a large population of astrocytes by ATP application potentiated neurotransmission in only ~40% of the recorded synapses further supports this hypothesis [46]. If this is the case, it would be interesting to test whether tripartite synapses are stable or dynamic functional units. The latter idea seems to be favored by the observation that coordinated structural changes in astrocytic processes and synaptic spines occur in hippocampal synapses [79] and in the somatosensory cortex where whiskers stimulation evoke morphological changes on astrocytic processes that cover synapses [80].

Box 2. Future questions

Regarding the properties and physiological consequences of the astrocyte–neuron communication, important issues remain largely unknown.

Among the general issues, key topics need to be further investigated:

(i) The molecular and cellular events underlying astrocyte–neuron signalling *in vivo*.

(ii) Role of astrocyte–neuron communication in brain function and animal behavior. While this communication is largely characterized at cellular and subcellular levels, what are its actual roles in neural network activity, brain function and animal behavior?

(iii) Role of astrocyte–neuron communication in brain pathology. Might the disruption of astrocyte–neuron signaling mechanisms result in brain diseases? Might this signaling lead to brain pathology and under what certain circumstances? Are astrocytes the appropriate cellular targets to direct therapeutic approaches for the treatment of some brain diseases?

To investigate these issues, new transgenic mice such as those designed to silence the molecular mechanisms involved in synaptically evoked astrocytic responses or in gliotransmitter release might be useful. Likewise, great help might be provided by transgenic mice that enable the selective stimulation of astrocytes *in vivo*, which is the strongest challenge that must be overcome to reveal the actual role of astrocytes in brain function and animal behavior.

Examples of particular questions are:

(i) What is the involvement of other neurotransmitter systems, such as dopamine or serotonin, on astrocyte excitability?

(ii) What are the specific properties of synaptic information processing by astrocytes in different brain areas? How are these properties regulated by different neurotransmitters?

(iii) Are different gliotransmitters co-released by single astrocytes? Are different gliotransmitters released by different astrocytes or by different astrocytic processes or domains?

(iv) What are the specific incoming inputs, the molecular mechanisms and the physiological conditions that govern the precise release of each gliotransmitter?

(v) Are tripartite synapses plastic elements? If so, what are the cellular signaling events and the molecular mechanisms that control the structural and functional plasticity?

The plasticity in the establishment of tripartite synapses might have strong impact on the function of the neuron–glia network. In any case, the fact that only a subset of synapses were effectively modulated by single astrocytes indicates that neuromodulation does not result from a wide spillover of the gliotransmitter but, instead, suggests the existence of specific signaling pathways between astrocytes and neurons, probably as a point-to-point form of communication.

Concluding remarks

Since the beginning of the 'glia revolution' in the 1990s, compelling evidence has been accumulated by many laboratories to firmly establish the concept of the tripartite synapse, in which astrocytes have functionally relevant roles in synaptic physiology. We know now that astrocytes are cellular processors of synaptic information and that they regulate synaptic transmission and plasticity. Consequently, astrocytes are involved in the processing, transfer and storage of information by the nervous system and, therefore, in addition to neurons, they must be considered as cellular elements involved in brain function. During recent years, a great advance has been produced in our knowledge of events underlying astrocyte–neuron interactions at cellular level, but it is apparent that we have only begun to appreciate the actual role of astrocytes in brain function and animal behavior (Box 2). A more comprehensive characterization of these cellular events and the actual impact of astrocytes on the activity of the neuron–glia network is still required. Finally, considering current evidence, it will not be surprising if future studies, in which the development of transgenic animal models will be fundamental, reveal important roles of astrocytes in different brain tasks and animal behavior.

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