Plasticity through Astrocytes: How the Glue becomes the Solvent.

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Short Summary
Astrocytes are known for their metabolic support of neurons, the only type of brain cell that was thought to communicate in the CNS. However, in this paper I will briefly describe different attributes that have recently been granted to astrocytes and their role in orchestrating the brain on terrains such as synaptic formation and stability, astroglial communication and their influence on synaptic plasticity.

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Introduction

Introduction Astroglia

In history, learning has always been done exclusively by neural cells. These cells, residing in the central nervous system (CNS) and periphery, are connected to each other and communicating with each other, being held in place by some kind of cement-like connective tissue called “neuroglia”[1] (*glia* is the Greek for glue). Among these glial cells, star-shaped cells were found[2], which were named ‘astrocytes’ for their stellate shape[3]. *Santiago Ramón y Cajal* did a great deal of research regarding astrocytes by introducing a new method of astroglial staining by glial fibrillary acidic protein (GFAP) and proposing physiological functions for astrocytes which were far ahead of his time. Cajal proposed involvement of glia in the supply of nutrients for neurons, as well as removal of neurotoxic substances and even in the regulation of synaptic activity[4]. Alas, he was unable to prove these functions due to the unavailability of advanced techniques. The outcome was that for the next century astrocytes became known as stagnant, passive cells that merely provide metabolic support for neurons. **Fig.1** shows an impression of the human brain anatomy, modified from chapter 2 of Neil R. Carlson’s Physiology of Behavior[5].

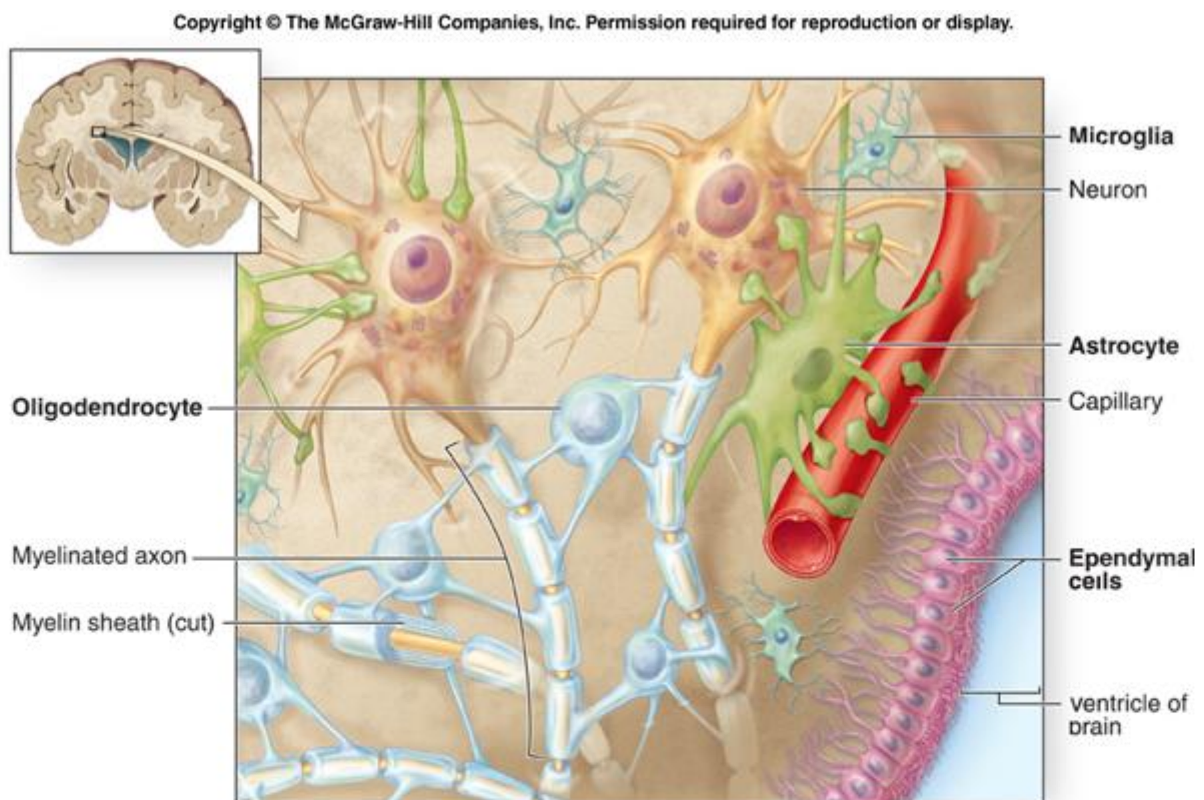

Fig. 1: The mammalian brain is an incredibly complex structure of neurons communicating with each other while being supported by glial cells. There are an estimated 100 billion neurons and 1 trillion glial cells in the human nervous system.

The glial cells can be divided in two general types: the macroglia class, which contains astrocytes and oligodendrocytes, and the microglia class, which acts like macrophages in the brain. Oligodendrocytes are similar to the Schwann cells, since both construct a sheath of myelin around neurons. While Schwann cells operate solely in the peripheral nervous system, oligodendrocytes are responsible for the myelination of axons in the central nervous system (CNS). Astrocytes, or astroglia, are the main point of interest in this review and. Most astrocytes position themselves between brain capillaries and neurons to provide neurons with oxygen and nutrients. You can see an astrocyte wrapping itself around a blood capillary with its ‘endfeet’, which are also known as pseudopodia or processes.

Since the development of new technologies in the last 20 years including confocal microscopy, dye-filling and fluorescent labeling, research in astrocytes has improved greatly, enabling researchers to get (real-time) close-ups of the cells and explore astroglia in detail. **Fig.2** shows the difference in visible structure between a micrograph of a GFAP[6] stained astrocyte and an image stained with erzin to show the intricate network between the processes which GFAP did not show[7].

Astroglial endfeet have shown to be much more detailed than thought before, with many more and much smaller processes, being able to contact multiple neurons, 300-600 dendrites and upwards of 10.000 synapses[8]. Each astrocyte forms a 'territory' or domain with brain capillaries adjacent to it. The domains of different astroglia do not overlap each other and form structural compartments regulated by single astrocytes[9].

Astrocytes are the most numerous cells in the human brain[10], but the ratio between astrocytes and neurons differs between species[9], with the highest number of astrocytes per neuron in humans. The size of a human astrocyte is roughly three times bigger than that of a mouse, and also the number of synapses in the functional domain of an astrocyte is much higher in comparison. Estimations of 90.000 synapses per murine astrocyte and around 2 million synapses per human astrocyte[11] show a large difference, but these numbers are probably very variable.

New insights in astroglial physiological functionality have appeared by the grace of new technology in addition to new anatomical information. This has provided a whole new field for investigations that has been expanding quickly in the last decade. Now astroglial physiology is one of the most exciting topics in neuroscience and it has changed the perception of brain functioning to great extent.

Instead of passive cells with metabolic functions, astrocytes have emerged as very dynamic cells with important and diverse functions. Functions include the communication between astrocytes and neurons or neighboring astrocytes, neural and synaptic growth, providing a stable environment for neurons and possibly regulating synaptic plasticity. These physiological aspects of astroglia will be described in this paper, emphasizing their implications on plasticity towards a view in which astrocytes orchestrate the central nervous system.

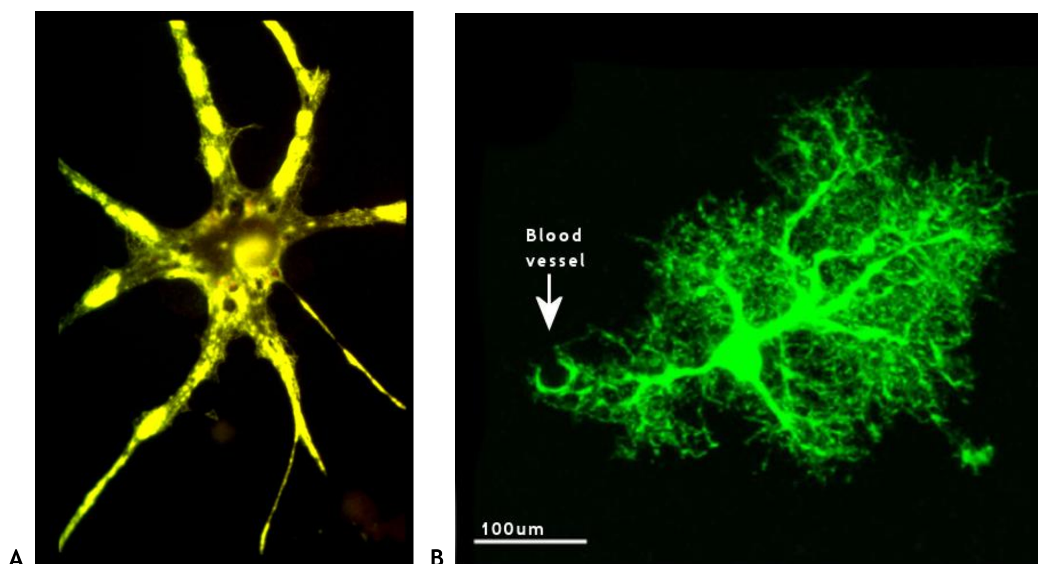

Fig.2: Micrographs of human astrocytes. **(A)** GFAP staining does not show the intricate network of astroglial processes **(B)** Single astrocyte of the mouse neocortex with numerous processes one of which contacting a blood vessel (left). The other processes end in thin lamellipodia- and filopodia-like terminals that enwrap synapses of adjacent neurons.

Astrocytes Provide a Stable Environment

Blood supply
For the normal functioning of neurons within the brain a special environment is necessary, this is created by separating the circulating blood from the cerebrospinal fluid (CSF). In the periphery, blood is contained by endothelial cells to form capillaries and other blood vessels. In the brain the endothelial cells of the capillaries need to be ensheathed by direct contact with the endfeet of the astrocytes to form tight junctions[12], which prohibit the exchange of many substances in the blood and endothelial cells to the CSF in the first place. Only very small, fat-soluble or nanoparticle coated[13] substances may cross the BBB, so only a very few drugs – about 5% of 7000 tested substances[14] - reach the CSF effectively. A few exceptional parts of the brain have an incomplete BBB, like the circumventricular organs, which have to be in direct contact with the blood to sense concentrations of various compounds, for example certain peptide hormones. When neurons become active, blood flow and glucose uptake increase locally to meet the local metabolic demand of oxygen and energy[15]. Astrocytes make this metabolic increase possible by vasodilatation and the resultant increase in blood flow is what can be made visible by functional MRI[16].

K^+ Homeostasis
The extracellular environment between neurons and glia is under a great influence of astrocytes. Potassium for example, is released whenever a neuron depolarizes during the repolarization phase and is usually quickly reabsorbed. When an entire nerve tract is activated the potassium concentration can rise more than 3-fold, what could interfere with the possibility of the nerves to depolarize again[17]. Pathological accumulation of extracellular K^+, for example during an epileptic attack[18], can lead to a disruption of functionality[19] (depolarization block) which can form a spreading depression that interrupts all neural activity nearby[20]. The removal of excess K^+ from the extracellular clefts is adapted locally to the requirements of the neurons[21] and is done by astrocytes instead of the (in)active neurons themselves[22]. One of these mechanisms is by spatial buffering, where excess K^+ is taken up locally by an astrocyte and dumped into ECS farther away by the same cell or neighboring glial cells trough gap junctions in a radial fashion, while the amount of K^+ that is taken up and released remains the same[23]. Other mechanisms for K^+ uptake are the net uptake of potassium by the Cl^-, K^+-cotransporter and the Na^+, K^+ -ATPase exchange.

pH Homeostasis
Most proteins, including enzymes and ion channels, are highly sensitive to protons and will undergo conformational changes when pH levels change. Therefore, it is important that the intra- and extracellular pH changes are kept in the relative narrow physiological range. During neuronal activity, large changes in pH may occur, especially when neurotransmission with γ-aminobutyric acid (GABA), glutamate or glycine is taking place. Changes in pH to acidic or alkaline have different consequences, but both change the electrical and synaptic activity of neurons. Astrocytes have many ways to counter pH changes, because of a variety of acid/base-transporting proteins in the plasma membrane and different ways of buffering which work in concert to restore the optimal physiological pH quickly[24].

Glutamate/GABA Homeostasis
Most excitatory transmission in the CNS is mediated by glutamate, which is the precursor of the main CNS inhibitory neurotransmitter γ-aminobutyric acid (GABA) and is a potent neurotoxin if not taken up quickly from the synaptic cleft. Most excitatory amino acid transporters type 2 (EAAT2) glutamate uptake transporters can be found on astrocytes[25], positioned between neighboring synapses. This positioning is necessary to prevent adjacent synapses to activate each other by spilling over. The neurons that release glutamate are, paradoxally, unable to synthesize glutamate and GABA *de novo* from glucose, due to the absence of pyruvate carboxylase[26]. Neurons would have trouble functioning without glutamate uptake and synthesis if the glutamate/GABA-glutamine cycle did not exist. Astrocytes do have pyruvate carboxylase, so they can convert glucose into glutamate. The glutamate synthesized *de novo*, and the glutamate taken up by astrocytes is converted into glutamine by glutamine synthetase. After that, glutamine is transported back to the neuron to be converted into glutamate again[27]. At GABA-ergic neurons, GABA largely follows the same paths, only the uptake of GABA is less privileged to astroglia because of the more ubiquitous distribution of GABA transporters (GAT)[28]. The GABA taken up by astrocytes is converted into glutamine and then transported to the neuron following the same route as glutamine made from glutamate[29].

To put it briefly, neurons in vivo would fail to function properly and most certainly perish without astroglial support.

Plasticity

Introduction Neural Communication
Neurons can conduct short-lasting electrical signals (action potentials) over a long distance at a high speed, and cause the transmission of signals to other neurons by neurotransmitters. Looking at **Fig.3** provides one with convenient images of the neural connection and summation of EPSPs leading to an action potential. Neurotransmitters on the postsynaptic neuron cause receptors to activate which in turn open channels, allowing ions to flow into the neuron, causing a change in transmembrane potential. This change in voltage is known as a postsynaptic potential (PSP), of which there are two types, the excitatory postsynaptic potential (EPSP) and the inhibitory postsynaptic potential (IPSP). EPSPs cause a neuron to depolarize, evoking an action potential if the depolarization is large enough, while IPSPs do the opposite. Summations of EPSPs cause an additive effect up to the point where the threshold is exceeded, thus inducing an action potential. The "strength" of a synapse is defined as the relative amount of change in transmembrane potential in response to the activation of the postsynaptic neurotransmitter receptors. (Modified from chapter 2 of Neil R. Carlson's Physiology of Behavior[5])

Early in the development of the brain, there is an overproduction of axons, causing more neural connections than necessary. Later in the development, the excess neural connections are lost by a mechanism called *pruning*. Pruning is a "use-it-or-lose-it" mechanism, where neural connections, or synapses, with sufficient activity are retained, while those that see too little activity are lost[30]. In other words, neural connections which are activated through experiences survive, so only experienced and well-used "strong" synapses will survive, while inexperienced and surplus "weak" synapses do not[31]. Synapses can become stronger or weaker in time and this process is widely accepted as the synaptic basis of memory and learning[32]. This variability of synaptic strength is called *synaptic plasticity*, which is an important part of this paper.

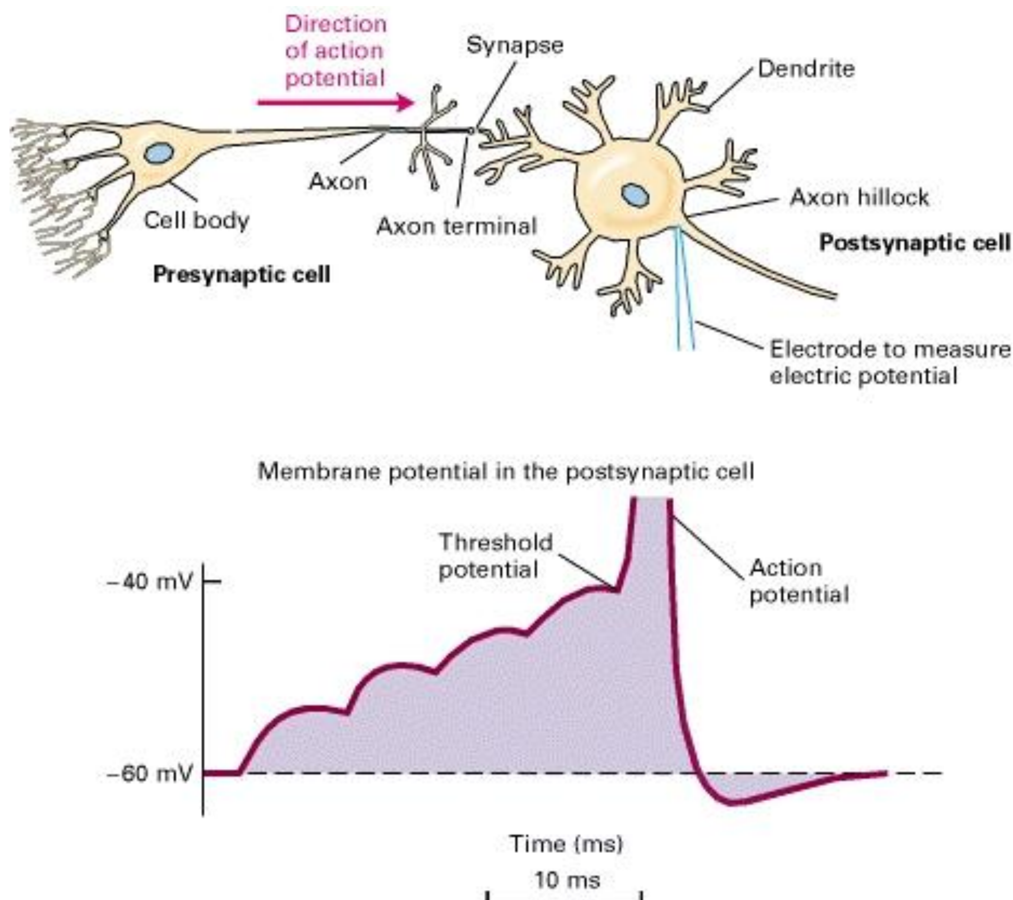

Fig. 3: The connection between neurons above and the additive effect of EPSPs in the synapse causing an action potential in the postsynaptic neuron.

Classic Plasticity

To understand neural plasticity, we have to start with the simplest learning mechanism known as 'classical conditioning', where a weak connection between neurons can become a strong connection. In this example a rabbit will be conditioned to an auditory tone followed by a harmless air puff into the eye. In a normal situation, a blinking reflex would follow a rapid air current to the eye for protection but hearing the tone would not cause blinking. In this experiment a 1000Hz tone will be heard for 10s, and in the last 500ms the puff of air will be blown into the eye. After a couple of repetitions of this protocol, the rabbit would blink in anticipation to the air puff, trying to protect its eye. Eventually the air puff is eliminated from the protocol, but the rabbit would still blink in the last second of hearing the tone. Classical conditioning has taken place, quite similar to how Pavlov, the discoverer of classical conditioning in the 1890s, made dogs drool by ringing a bell. When classical conditioning takes place, what kinds of changes occur in the brain? In this example, we assume that only three neurons are needed in this circuit: one for the detection of the air puff, one for the auditory tone and one for blinking. In reality learning something like this would involve thousands of neurons, but let's see how this theoretical circuit works. If we present a tone, the rabbit would not react, because the auditory neuron has a very weak connection to the blinking neuron. The excitatory postsynaptic potential (EPSP) it produces is too low to cause a depolarization. If during the time of the tone a stimulus (the air puff) would cause the blinking neuron to fire, both neurons would become associated with each other and the weak signal (tone) would become strong enough to cause blinking by itself after enough repetitions of this protocol. (Modified from chapter 13 of Neil R Carlson's *Physiology of Behavior*, 9th edition[5])

This mechanism was revealed by Donald Hebb in 1949 by stating: "The general idea is an old one, that any two cells or systems of cells that are repeatedly active at the same time will tend to become 'associated', so that activity in one facilitates activity in the other.", which lead to his more well-known statement: "Cells that fire together, wire together."

Nobel laureate Eric Kandel was a pioneer in this field, his results provided solid evidence for the mechanistic basis of learning as "a change in the functional effectiveness of previously existing excitatory connections", by researching habituation in sea slugs[33]. By using a, compared to mammals, simple organism, he was able to lay the foundations for the research of long-term potentiation.

Long-term Potentiation

The way a synaptic connection is made stronger, and what Hebb meant with 'wiring together', is by the process of long-term potentiation (LTP). There are many different types of LTP[34], but only the CA1 hippocampal LTP, dependant on the NMDA receptor (NMDA-R) will be described here. In such a synapse, a series of pulses delivered at once in a high frequency causes a summation of multiple excitatory postsynaptic potentials (EPSP), which depolarizes the postsynaptic membrane. Meanwhile, glutamate is released from the presynaptic neuron which binds to the NMDA receptor, along with D-serine, an endogenous ligand for the glycine binding site, causing the NMDA-R to activate. Because of the depolarization of the neuron a Mg^{2+} "plug", clogging the calcium channel of the NMDA receptor, is expelled, which allows a rapid influx of calcium ions to enter the postsynaptic neuron[5]. The increase of calcium concentration triggers a short-lasting activation of several proteins that mediate the maintenance of long-term potentiation. View **Fig. 4a** to see the results from an experiment increasing the post-synaptic membrane potential by D-Serine, against an NMDA receptor antagonist, modified from ref.35.

Especially important for LTP are several protein kinases, including protein kinase C (PKC)[36], calcium/calmodulin-dependent protein kinase II (CaMKII)[37], mitogen-activated protein kinase (MAPK)[38] and protein kinase A (PKA)[39]. These protein kinases are necessary to maintain LTP by improving the function of AMPA receptors (AMPAR), that increase their permeability to Ca^{2+} due to phosphorylation by the protein kinases[34]. More AMPARs are congregated to the postsynaptic membrane by AMPAR trafficking[40] and increased AMPAR synthesis[41] because of the protein kinase activation. As a reaction to LTP, dendritic spines increase in size, except for already grown spines that do not react to LTP anymore[42]. This may suggest a mechanism of memory storage, where information on large dendritic spines is protected from further potentiation by readout[43]. Thus, through long-term potentiation, the postsynaptic synapse is strengthened. This means that, when activated, the synapse is able to cause a depolarization quicker than before the occurrence of LTP.

When weak stimulation of a certain synapse is insufficient for the induction of LTP, simultaneous strong stimulation of another synapse nearby will induce LTP at both synapses. This process is called *associativity*, and it is one of the most important mechanisms in which two different signals are linked together.

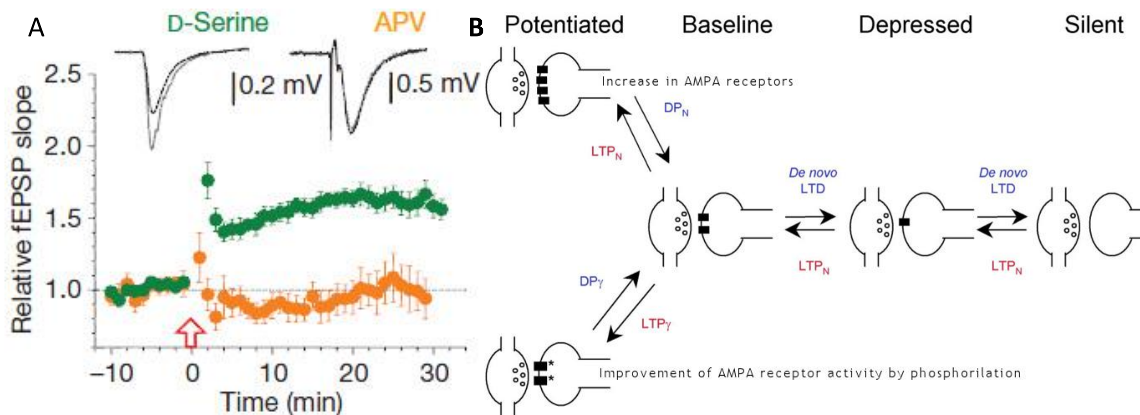

Fig. 4: Long-term potentiation. **(A)** The effect of D-Serine on EPSP slope (green) against the NMDA receptor antagonist (2R)-amino-5-phosphonovaleric acid (APV)(Orange). **(B)** The different states of synaptic potentiation. Rectangles represent AMPA receptors.

Long-term Depression

Long-term depression (LTD), the process of weakening a synaptic connection, is the opposite of LTP but uses more or less the same mechanisms. LTD is caused by low frequency stimulation during a certain time-span, instead of a high frequency during a short period what causes LTP[44]. LTD is also dependant on the NMDA receptor and there is evidence that LTP has a higher threshold than LTD for NMDA receptor activation and subsequent Ca^{2+} influx. The amount of Ca^{2+} influx from NMDA-Rs is one of the critical factors determining whether LTP or LTD is induced[45]. High level cytoplasmic Ca^{2+} for a brief duration may be critical for LTP induction, but a moderate Ca^{2+} level for a sustained duration may be critical for LTD induction[45]. LTD causes dephosphorilation of CaMKII by activation of type 1 protein phosphatase (PP1)[46]. LTD induces a decrease in AMPA receptors, by their removal from dendritic spines in endocytotic vesicles[47]. Long-term depression causes a synapse to be more difficult to depolarize. Also, LTD has an important function in a process called *metaplasticity*, in which strong synapses, potentiated by LTP, are weakened to be able to be potentiated later again. If there was no homeostatic weakening of synapses, there would be a point in time that most synapses are potentiated by positive feedback from LTP and stay that way, causing a static brain that is unable to learn. LTD provides negative feedback, so synapses that have been potentiated will be weakened after some time, enabling LTP to occur again. For a schematic view of the different states of synaptic strength, view **Fig. 4b** (taken from ref. [48]).

Silent Synapses

Long-term potentiation is now thought to be linked with the un-silencing of ‘silent’ synapses[50]. Silent synapses show an excitatory postsynaptic current (EPSC) when depolarized, because of the presence of NMDA receptors. However, a silent synapse lacks EPSCs at the resting membrane potential, probably due to a lack of AMPA receptors (AMPAR) which could conduct currents at resting membrane potential. Absence of functional AMPARs renders a synapse ‘silent’, unable to mediate synaptic transmission under physiological conditions[49]. Silent synapses exist almost everywhere in the brain, but the interesting part is that induction of LTP could ‘unsilence’ these synapses[50]. Keep in mind that a ‘silent’ synapse is not the same as a synapse less active by LTD and that ‘unsilencing’ is not the same as LTP, because LTP is also possible on ‘active’ synapses.

In the first days after birth, virtually all hippocampal synapses are silent and roughly half have become activated by the second to third post-natal week[51]. Anatomical evidence shows an increase in AMPARs during development, while NMDA-Rs remain constant[52]. This would mean that during spine maturation NMDA-R expression occurs first and the recruitment of AMPARs occurs later. There is evidence that NMDA-Rs actively keep AMPARs away from immature spines until an appropriate signal triggers their recruitment[53]. The molecular mechanisms of AMPAR recruitment induced by NMDA-R activation in silent synapses are still largely unknown, but ‘unsilencing’ of neurons is obviously an important part of initial plasticity and learning.

In this chapter, it has been discussed that synaptic plasticity is controlled by transmitters reaching postsynaptic membranes. Neurotransmitters can have strengthening, weakening and awakening effects on neurons throughout the CNS. The bigger picture of when plasticity is triggered remains elusive when only neurons are taken in account.

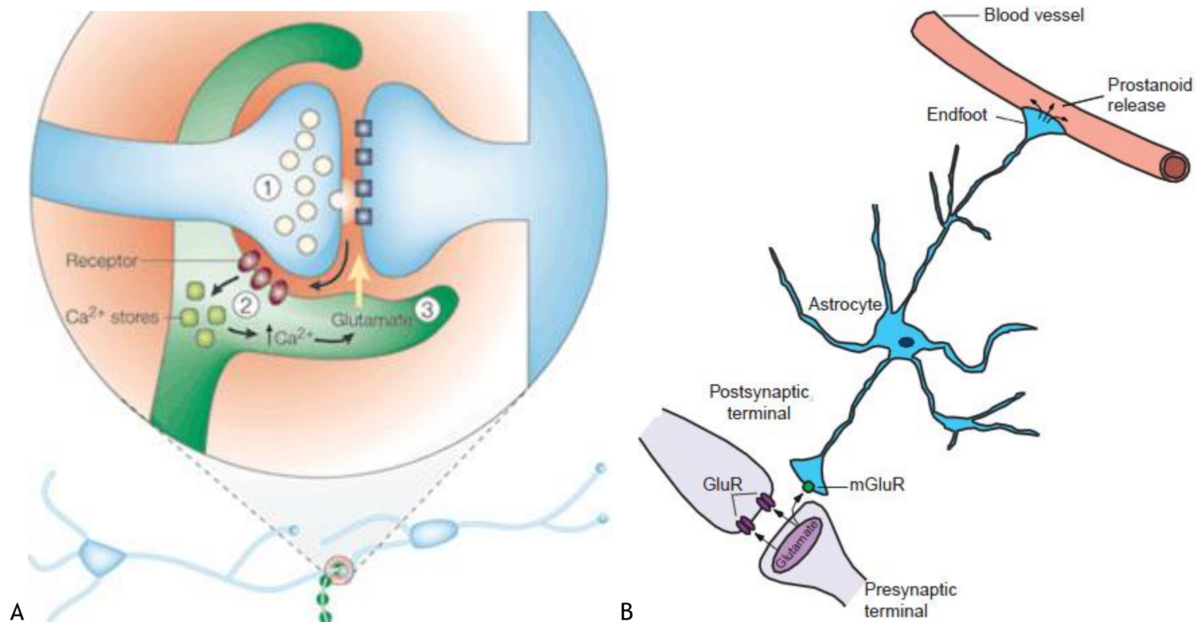

Fig. 5: Tripartite synapses **(A)** A conceptual drawing of an astrocyte enwrapping the pre- and postsynaptic neuron **(B)** A drawing of an astrocyte positioning itself between a synapse and a blood vessel.

Tripartite Synapse

It was thought for a long time that a synapse, the connection between nerve cells, consists of only two parts, the presynaptic and postsynaptic part, that were able to communicate with each other. The concept of 'tripartite synapse' emerged during the 1990s when many laboratories discovered bidirectional communication between neurons and astrocytes. In this concept astrocytes exchange information with the pre- and postsynaptic neurons in response to synaptic activity.

In **Fig.5a** the tripartite synapse is shown where you can see the astrocyte enwrapping or ensheathing both the pre- and postsynapse (modified from ref. 95), **Fig.5b** shows the positioning of such a tripartite synapse[54]. This way, the astrocyte can receive signals from the synaptic cleft and release factors to the pre- and postsynapse. Factors include 'gliotransmitters' for communication, which will be discussed later in this review, and factors involved in synapse formation, which will be described below.

One of the main functions of the astroglial tripartite synapse is the *on demand* supply of energy to active neurons. View **Fig.6** on page 9 for a convenient visualization, modified from ref.55. It has been proposed that neurons obtain most of their energy from extracellular lactate, a glucose metabolite produced by astrocytes. Astroglia position themselves between the vasculature and the neurons, supporting the concept of metabolic coupling between glia and neurons. Lactate is transported by monocarboxylate transporters from the astrocyte to the synaptic terminal as a source of neuronal ATP[56]. Astroglial networks are able to transport glucose and lactate trough connexin gap junctions between astrocyte. Release of glutamate from the synapse increases this glucose trafficking by activation of AMPA receptors[57]. These studies would suggest that synaptic activity regulates astroglial metabolism, which consequently feeds these active neurons with lactate[58].

Furthermore, astroglial processes are not fixed to one location. Instead, they are able to display dynamic remodeling[59]. Exposure to glutamate induces an increase in the number of endfeet on astrocytes in culture[60]. On the other hand, visible changes in the shape of dendritic spines, the small protrusions on dendrites that receive the majority of postsynaptic signals, can occur within seconds[61]. This suggests that synapses can undergo rapid anatomical plasticity. It is tempting associate astroglial movement with synaptic morphological and functional changes.

Since astroglial processes position themselves directly around a synapse and are able to respond to synaptic activity, the role of astrocytes in regulating synaptic strength may be quite important. As described in detail in the following chapters, astroglia are able to release substances for synapse formation and strengthening. D-serine for example, the endogenous ligand for the NMDA receptor, is released solely by astrocytes and is necessary for long-term potentiation[62].

Astrocytes in Synapse Formation

The traditional perception on the process of synaptogenesis solely as a capability of neurons has been changed by research over the past decade. When it was shown that the CNS synapse number was profoundly regulated by non-neural signals, and that astrocytes play an important role in the induction and stabilization of CNS synapses[63], the possibility of active participation by glia in synaptic plasticity emerged. More and more it became established by different laboratories that astrocytes provide instructive signals to control the development and formation of synapses.

Evidence emerged in vitro, when researchers tried to culture purified rodent retinal ganglion cells (RGCs). RGCs are a useful model system to investigate the role of astrocytes in synapse development, because RGCs can be immunopurified and cultured for several weeks in the absence of glia and other cell types[64]. RGC neurons show little spontaneous synaptic activity unless they are cultured with an astrocyte feeding layer or astrocyte-conditioned medium (ACM). Only a few factors secreted by astrocytes or by direct contact have shown improvements in synaptic activity and synaptogenesis in RGC neurons so far.

Although both astrocytes and neurons contain cholesterol synthesis proteins, it appears that neurons rely, at least a little, on the production of cholesterol by astrocytes in culture. In a RGC culture study of Pfrieger et al, cholesterol in complex with apolipoprotein E (ApoE) was released from astrocytes and increased the number of functional glutamatergic synapses. When cholesterol was applied directly to neurons in culture, spontaneous synaptic activity increased as well as the amount and efficacy of synaptic vesicle release[65]. Cholesterol is an essential component for exocytotic vesicle production and serves as a limiting factor in vesicle formation[66]. During development, as hypothesized by Pfrieger, neurons may reduce or even abandon cholesterol synthesis and depend on cholesterol produced by astrocytes, transported by a cholesterol 'shuttle', to save energy for other processes[67].

Thrombospondins (TSPs) 1 and 2, extracellular factors produced by astrocytes, have been shown to increase the number of excitatory synapses in culture by Barres et al. When directly applied to RGC cultures, TSP1/2 increased the number of synapses almost 3-fold. TSP1/2 is highly expressed in the developing brain, but it is downregulated in the adult brain, raising the possibility of switch-like properties to activate synaptogenesis. Synapses induced by TSPs are postsynaptically silent, although they look like normal synapses when examined by electron microscopy. When astrocyte feeding layers are added, the synapses are able to become fully active, therefore TSP1/2 is just one of the factors necessary for synaptogenesis[68]. Although TSP1/2 activity is associated with brain development, its necessity has been shown in recovery of a stroke. When TSP1/2 knockout mice had to recover motor functions after a brain infarct, the TSP1/2 KO mice showed significant synaptic density and axonal sprouting deficits, along with impaired recovery[69].

One of the main events of synaptic plasticity is the trafficking of AMPA receptors to synaptic sites. Now evidence has emerged that factors secreted by astrocytes modulate AMPAR trafficking. Astroglial tumor necrosis factor alpha (TNF-α) specifically mediates AMPAR trafficking, but not other glutamate receptors, to the postsynaptic membrane. Inhibition by capturing released TNF-α with soluble TNF-α receptors reduces AMPAR trafficking[70].

Although soluble factors play roles in the forming of synapses, direct astrocyte-neuron contact is necessary to form synapses that are receptive to incoming signals[71]. The protein kinase C (PKC) pathway, activated by contact with astrocytes, plays a certain role here, because inhibition of PKC signaling disrupts the co-localization of synaptotagmin1 (a presynaptic marker) and PSD95 (a postsynaptic marker) and prevents excitatory postsynaptic potentials[72]. A recent study has shown that astrocyte-neuron contact with γ-Protocadherins (γ-Pcdhs) is critical for synaptogenesis in developing cultures. γ-Protocadherins are early cadherins, which mediate cell-to-cell adhesion, and have recently been linked to activation of certain pathways, but none have been confirmed in astrocytes. However, restricted mutation of the astroglial Pcdh-γ cluster in vivo significantly delays excitatory and inhibitory synapse formation, revealing the first known contact-dependent mechanism by which perisynaptic astrocyte processes promote synaptogenesis[73].

From this evidence, I would conclude that astrocytes are morphological dynamic cells with an important modifying character towards synaptogenesis and synaptic strength due to intimate relationships with synapses.

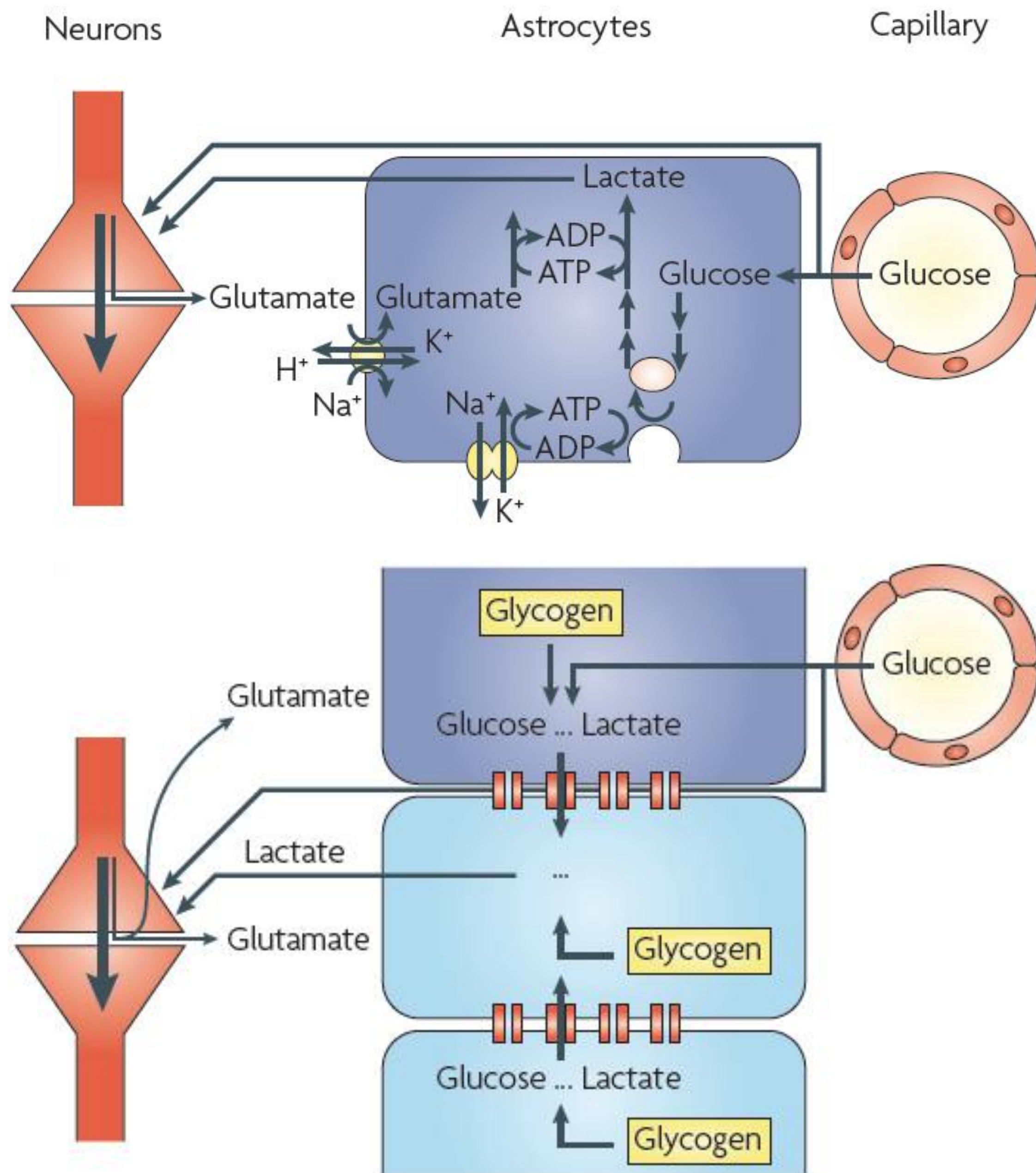

Fig. 6: Metabolic support from blood vessel to synapse. **(A)** Astroglial lactate is transported towards the synapse in response to neural activity. **(B)** Glucose and lactate are able to diffuse between astrocytes via connexin gap junctions.

Calcium Waves

Calcium Homeostasis
In astrocytes, the difference in calcium concentrations between extracellular (~1mM) and intracellular (~100nM) is a factor 10.000, so the intracellular calcium concentration is tightly regulated within narrow limits. To keep excess Ca^{2+} out of the cytosol, the plasma membrane calcium pump (PMCA), mitochondrial membrane calcium pump (MMCA), NCX and sarco-endoplasmic reticulum CA ATPase (SERCA) transport excess Ca^{2+} back to the extracellular space or into mitochondria and ER as shown in **Fig.7**, taken from ref. 74. All these transporters use ATP as energy source for active transport, except NCX, the main pathway for calcium efflux that works in concert with the NA/K-ATPase transporter, which creates a K^+-gradient over the cell membrane, enabling the efflux of Ca^{2+} to the extracellular space[74].

Sometimes Ca^{2+} influx from the extracellular space is required, when internal Ca^{2+} stores are (nearly) empty and need to be replenished, for example when the ER is emptied by IP_3R activation or SERCA inhibition. This calcium influx, known as capacitative Ca^{2+} entry, is mediated by store operated calcium channels (SOC), where calcium release activated calcium channels (CRAC) and transient receptor potential proteins (TRCP) are involved in the influx of extracellular calcium[75]. Also voltage-gated calcium channels (VGCC) induce a Ca^{2+}-influx when a depolarization occurs, or with high extracellular K^+ concentrations[76].

Astroglial calcium concentration can also increase through release from internal stores, especially the endoplasmic reticulum (ER) and mitochondria. The release of calcium from ER stores is mediated by activation of inositol-1,4,5-triphosphate receptors ($Ins(1,4,5)P_3R$ or IP_3R), usually when IP3 is produced by phospholipase C (PLC) in response to activation of G-protein-coupled receptors (GPCR), or by activation of caffeine/ryanodine receptors (RyR)[77].
Mitochondria also release and take up calcium in a buffering manner. These organelles are able to accumulate and release large quantities of calcium[78]. The role of mitochondria on Ca^{2+} homeostasis is thought to be on a much larger scale than buffering only. (for reviews: [79]) In short, Ca^{2+} release from mitochondria is quite complex, but it would suffice to say that mitochondria are a source of intracellular Ca^{2+}.

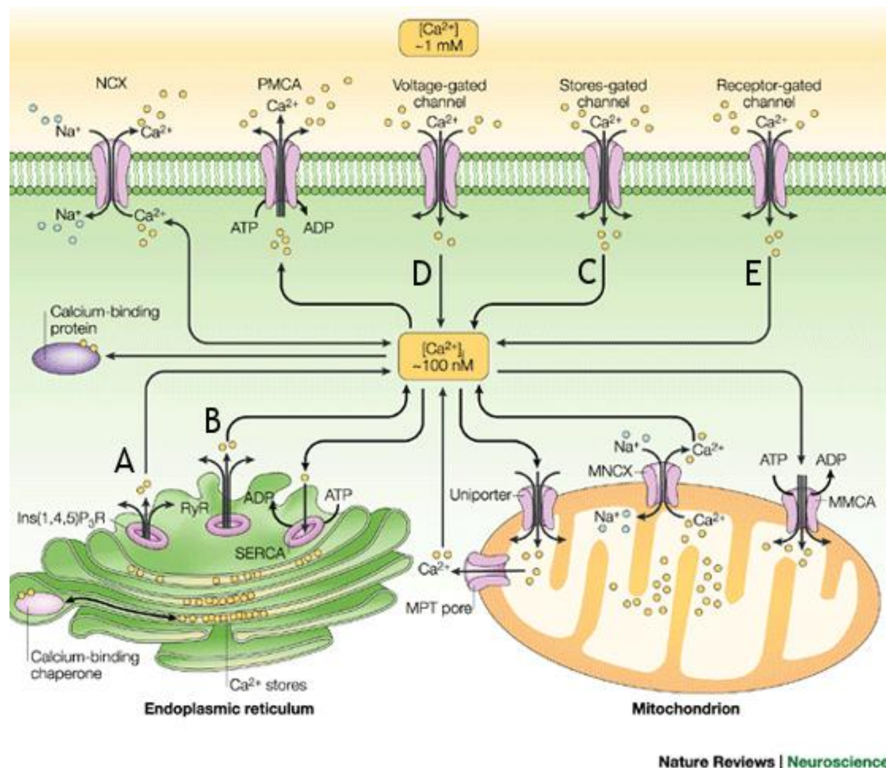

Fig.7: Sources of calcium for astrocytes and their mechanisms mediating intracellular calcium concentrations. The letters indicate processes of calcium influx to the cytosol, which are described below.

Astroglial Calcium Sources

Utilize **Fig.7** as visual reference to this paragraph, because some processes are marked on the picture with a letter like this **[X]**.

Most of the calcium needed for Ca^{2+} waves and Ca^{2+}-dependent exocytosis is released from the endoplasmic reticulum (ER). Hua and others have shown that the greatest reduction in glutamate release is achieved, by depleting the ER of its calcium using the SERCA calcium re-uptake inhibitor thapsigargin. **[A]** Inositol 1,4,5-triphospate (IP_3) seems to be responsible for the calcium release from the ER, which is based on the opening of CA channels by specific activation of ionotropic IP_3-receptors in the ER. Indeed, antagonism of the IP_3 receptor by intracellular Diphenylboric acid 2-aminoethylester (2-APB) greatly reduced glutamate release from astrocytes[80]. The receptors that activate the IP3 pathway include the metabotropic glutamate receptor subtype 1 and 5 (mGluR) and metabotropic purinergic receptor P2Y1 and P2Y2[81].

[B] Another possible mechanism for Ca^{2+} release from ER is the activation of caffeine- or ryanodine receptors (RyR) which has been reported in astrocyte cultures by Hua et al. In situ however, RyR activation does not show much Ca^{2+} release, even questioning the existence of astroglial ryanodine receptors in vivo[82].

Mitochondria also act as intracellular calcium stores. It has been shown that blocking calcium accumulation by Ca^{2+} uniporters in mitochondria raises intracellular Ca^{2+} and increases glutamate release[83]. On the other hand, mitochondria modulate intracellular Ca^{2+} levels during Calcium waves by buffering the Ca^{2+} levels. Inhibition of mitochondrial Ca^{2+} uptake had significant effects on intra- and intercellular calcium wave propagation[84]. **[C]** Mitochondria also seem to actively regulate Store Operated Calcium Entry (SOCE), which affects repetitive Ca^{2+} responses induced by mechanical stimulation and ATP additions[84].

Obviously astrocytes take up and extrude Ca^{2+}ions from the extracellular space to maintain internal Ca^{2+} stores. Furthermore, astrocytes accumulate calcium through mechanisms that support calcium wave propagation and transmitter secretion. Evidence for this came from Hua et al., who showed that blockade of membrane calcium channels by extracellular Cd^{2+}, a Ca^{2+}-channel blocker, caused a significantly lower release of glutamate by astrocytes. Many other mechanisms that mediate Ca^{2+} influx from the extracellular space to the astrocytes have been reported:

- **[D]** Voltage Dependent Calcium Channels (VDCCs) respond to neural depolarization induced high extracellular K^+ levels and cause Ca^{2+} influx and glutamate release[85].
- It seems that in astrocytes, capacitative Ca^{2+} entry is not causing TRPC opening, because during capacitative Ca^{2+} entry the TRPC-permeable ions Sr^{2+} and Ba^{2+} do not enter the astrocyte. TRPC3 activation with exogenous oleyl-acetyl-glycerol (OAG), a structural analog of DAG, causes intracellular, IP_3 independent, Ca^{2+} oscillations. This implies a novel mechanism of Ca^{2+} entry from the extracellular space[86]. Blocking TRPC1 reduced Ca^{2+}-dependent glutamate release, indicating that Ca^{2+} entry through TRPC1 channels contribute to Ca^{2+} signaling in astrocytes[87].
- Another way for Ca^{2+} to enter the cytosol is via the purinergic P2X7 ionotropic receptor[88]. **[E]** However, specific inhibition of P2X7 receptors by brilliant blue G had no effect on mechanically induced Intercellular Ca^{2+} Waves (ICW)[84], suggesting that P2X7 receptors are not involved. Instead, PLC-linked metabotropic P2 receptors, most likely P2Y2, are responsible for ICW propagation induced by mechanical stimulation[84]. Thus, metabotropic purinergic receptors (P2Y) show increase in intracellular Ca^{2+} by inducing IP3-mediated release, but more research is needed to show Ca^{2+}-increase through ionotropic purinergic receptor (P2X) activation.

Single Cell Calcium Transient

The definition of a calcium wave, or calcium transient when intracellular, is the localized increase in cytosolic Ca^{2+} that causes a progression of similar events in a wave-like manner. These calcium waves can be intracellular, restricted to one cell, or intercellular, causing calcium waves in neighboring cells[89]. The basic sequence that leads to intracellular Ca^{2+} waves in astrocytes usually involves activation of G-protein-coupled receptors (GPCR), which cause phospholipase C (PLC) to produce IP_3. IP_3R activation leads to Ca^{2+} release from the endoplasmic reticulum (ER)[80]. Calcium wave propagation throughout the cell is caused by a four component mechanism based on the released Ca^{2+}, two of the mechanisms are for Ca^{2+} amplification and two are for negative feedback:

1. The activation of nearby IP_3Rs due to co-agonistic action of Ca^{2+} on these receptors[90].
2. The additional generation of IP_3 through the Ca^{2+}-dependent activation of PLC[91].
3. The buffering through Ca^{2+} uptake by mitochondria to reduce Ca^{2+} concentrations[92].
4. The control of Ca^{2+} diffusion speed through single astrocytes, by low affinity Ca^{2+} buffers (calcium binding proteins) which bind Ca^{2+} ions[93].

Intercellular Calcium Waves

At first, propagation of calcium waves was thought to be mediated by inositol 1,4,5-triphosphate (IP_3) moving through gap junctions to the next astroglia, because IP_3 antagonism by heparin prevented this movement[94]. Other groups have proposed gap junctions as the channels in which calcium waves can spread to neighboring cells (for review: Haydon, 2001, ref.95) It seems that connexin-43 hemichannels are the proteins responsible for adjacent intercellular communication by Ca^{2+} flow through gap junctions[96]. It is impossible that intercellular Ca^{2+} wave propagation by gap junctions is the only mechanism, because it has been shown that calcium waves are able to cross a cell free area up to 120 µm[97]. For this, ATP has been identified as extracellular messenger, and is released to the extracellular space during Ca^{2+} wave propagation. When the purinergic antagonist *apyrase* is present in the extracellular space, calcium waves are reduced or even abolished[98]. **Fig.8** shows the conceptual drawings for CA wave propagation[95], and **Fig.9** shows an artificially colored wavefront of CA wave propagation in culture[99].

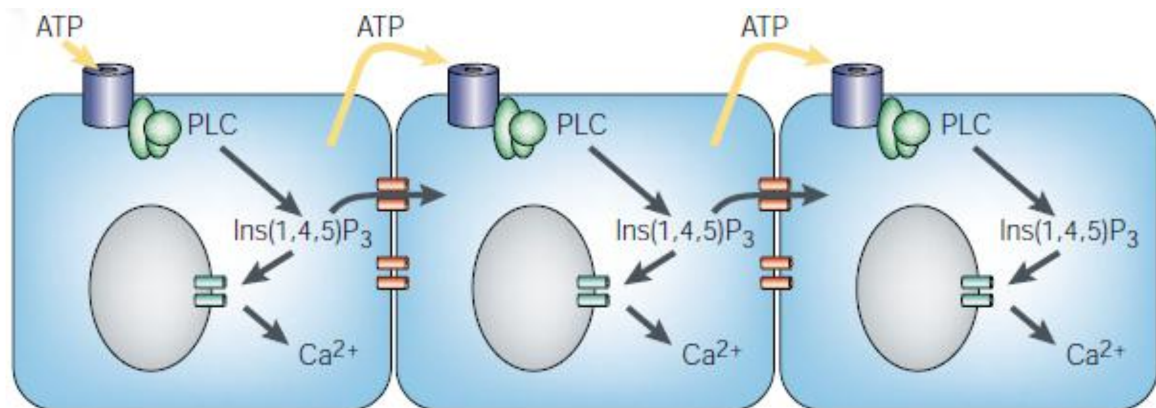

Fig.8: Putative mechanism for glial Ca2+ wave generation. Ca2+ is released from internal stores in response to elevated internal inositol-1,4,5-trisphosphate (Ins(1,4,5)P_3). Ins(1,4,5)P_3 can diffuse to neighboring cells through gap junctions to cause short-range signaling. Longer range calcium signaling requires the release of ATP, which causes the regenerative production of Ins(1,4,5)P_3 and further release of ATP from neighboring astrocytes.

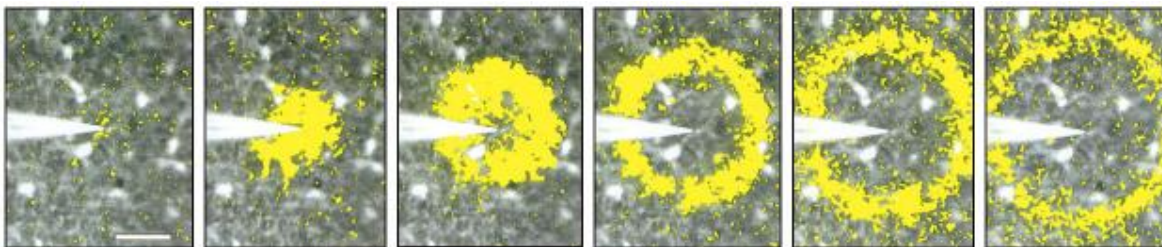

Fig.9: Spread of a Ca21 wave initiated by a mechanical stimulus. The fluorescence
image is shown in black and white. Superimposed yellow rings mark the leading edge of the Ca21 wave (where the change in fluorescence between successive panels exceeded a threshold value). Interval between panels, 0.93 s.[1] scale bar = 50 um.

Physiological Relevance

While both intracellular and intercellular calcium waves must have their functions, it is difficult to translate changes in calcium ion concentrations into physiological effects without getting ahead of the facts. Nonetheless, valuable insight has been acquired as an outcome of calcium wave research. At first we have to realize that not only neurons are the only cells in the CNS able to conduct signals and communicate with each other. Astroglia communicate much slower if you look spatio-temporally, but valuable information can be conveyed between astroglia or astrocytes and neurons as well.

An example of the function of a single cell calcium transient is the dynamic regulation of blood flow in response to neural activity. When an astrocyte receives glutamate from the overspill of an active synapse, Ca^{2+} concentrations are locally increased with a calcium transient as a result[100]. The calcium transient then surges toward the nearest blood vessel, causing local vasodilatation thus increases in oxygen and nutrient availability and uptake[101]. These nutrients can even pass from astrocyte to astrocyte through gap junctions to synapses in need for metabolic support[58].

Also, rapid spontaneous motility was reported to occur in astroglial processes close to active synaptic terminals, a location which is likely to evoke calcium increases in astrocytes by the activation of glutamate receptors[102], so changes in intracellular calcium concentration could be associated with rapid motility and morphological changes of astrocytes. It would be tempting to connect this evidence with the possibility that astroglial processes move towards synapses with high activity to provide feedback and support for that synapse.

Calcium-dependent exocytosis is one of the most important and best researched consequences of astroglial calcium signaling. This process will be extensively reviewed in the chapter below. In the hippocampus, observations were made that single astrocytes can release glutamate onto adjacent neuronal processes, thereby controlling simultaneously the excitability of several neighboring pyramidal cells by releasing slow currents of glutamate onto NMDA-Rs of multiple synapses [103, 104]. The synchronization of synapses that may be the result of these simultaneous glutamate releases may mediate the 'wiring together' of synapses as in long-term potentiation. A single astrocyte may be the regulator of a very small network within the brain by uniting a group of synapses together. In my opinion these synchronizations could be the key to thinking of something, by the connection of multiple attributes that contribute to the concept of a single thought or idea.

But before I get ahead of the facts, the effects of astroglial calcium waves on plasticity are only speculative at this moment. Obviously, if astrocytes mediate synaptic plasticity by means of gliotransmission, synchronization and motility, calcium waves will play an important role in astrocyte functioning. The exploration of brain functioning has taken a big leap by the evidence of astroglial communication, but many more years of research are needed to understand this properly.

Gliotransmission

Introduction Gliotransmission

Release of “gliotransmitters” by astrocytes was not known before 1976[105], since then it has been an important research subject in neuroscience. According to Volterra et al[106], one may call a substance a ‘gliotransmitter’ if it fits the following definitions: A gliotransmitter is synthesized by and/or stored in glia, released when triggered by physiological and/or pathological stimuli, able to activate rapid (milliseconds to seconds) responses in neighboring cells and is involved in (patho)physiological processes. Gliotransmitter release has been connected with chronic pain[107], synaptic plasticity[108], neuroinflammation[109] and neurodegeneration[110], but from these equally interesting topics, only synaptic plasticity will be described in this paper.

Neuron to Astrocyte Communication

Nowadays it has been well established that astrocytes can respond to neuronal activity[111]. Most receptors for different neurotransmitters that are found on synapses have been reported to be expressed by astrocytes as well. Both types of glutamate receptors, ionotropic (AMPA, kainate) and metabotropic (mGluRs) are well expressed and even astrocytic expression of NMDA receptors has been shown[112]. Other receptors found on astrocytes include the GABA-A and -B receptors ($GABA_AR$, $GABA_BR$), purinergic receptors for adenosine and ATP, acetylcholine receptors (nAChR, mAChR), α1- and ß2-noradrenergic receptors, as well as receptors for serotonin (5-HT), dopamine (DA), opioids, oxytocin, vasoactive intestinal peptide (VIP), histamine and many others[113]. As probably all receptors expressed by astrocytes have their own physiological role, some of those will be described in more detail below, it would suffice to say that astrocytes react to a broad spectrum of transmitters released in the brain.

It has been observed that receptors co-localize near the synaptic release site[114], suggesting an underlying structure for the placement of receptors. Indeed, surface organization by lipid raft-like ultrastructures has shown to be essential for successful calcium wave propagation[115] and plays a role in glutamate exocytosis[116]. In response to glutamate for example, astrocytes will increase the intracellular Ca^{2+} concentration, which may lead to calcium waves or Ca^{2+}-dependent exocytosis.

Ca^{2+}-Dependent Exocytosis

Similar to neurons, also astrocytes possess the complete exocytic apparatus for the construction and release of secretory vesicles known as the “soluble N-ethylmaleimide-sensitive factor (NSF)-associated protein receptors” (SNAREs). The essential proteins for v- and t-SNAREs were discovered in astrocytes, along with V-type H(+)ATPase, which creates the proton concentration gradient necessary for glutamate transport into vesicles, and the three isoforms of vesicular glutamate transporters (VGLUT(1,2,3)) which use the photon gradient made by the V-ATPase to pack glutamate into vesicles[117]. See **Table 1** for detailed information on the secretory proteins and their discoverers.

The secretory mechanism for these vesicles is mediated by increases in Ca^{2+}concentrations, in a manner as shown in **Fig.10,** taken from (A) ref.[118] and (B) ref.[119]. There are many factors that increase intracellular Ca^{2+}concentrations, but the main mechanism for vesicle release is the activation of inositol 1,4,5-triphosphate (IP_3) by phospholipase C[80]. Other mechanisms that increase intracellular Ca^{2+} are most likely also involved in Ca^{2+}-dependent exocytosis[120].

At this point, not much can be said about the mechanisms of astroglial Ca^{2+}- and SNARE-dependent exocytosis, because instead of secreting only one kind of vesicle, astrocytes contain and possibly secrete by means of various types of organelles which have been proposed to be important in exocytosis of gliotransmitters[121]: synaptic-like microvesicles (SLMVs)[122], dense-core granules[123], lysosomes[124] and extra-large organelles with a diameter of several µm[125]. Such heterogeneity has not been taken in account in past research, so earlier descriptions of astrocytic secretions are probably not fully accurate, due to mixed contributions of different exocytic organelles[121]. Ca^{2+}-dependency of vesicle release remains certain, regardless of vesicle size. How and when different types of vesicles are released is something for further research.

Table.1: *Proteins of the Exocytic Apparatus Expressed in Astrocytes.* Modified from Neuroglia 2nd edition

Type of Protein	*Role in Exocytosis*	*References*
Synaptobrevin II (VAMP2)	v-SNARE	Maienschein et al. (1999)
Cellubrevin (VAMP3)	v-SNARE	Maienschein et al. (1999), Bezzi et al. (2004)
TI-VAMP	v-SNARE	Calegari et al. (1999)
Syntaxin 1	t-SNARE	Calegari et al. (1999), Maienschein et al. (1999)
SNAP23	t-SNARE	Calegari et al. (1999)
SNAP25	t-SNARE	Maienschein et al. (1999)
Synaptotagmin I	Ca^{2+} sensor	Maienschein et al. (1999)
Synapsin I	Cytoskeleton-vesicle interaction	Maienschein et al. (1999)
Rab3 (a and b)	Vesicle trafficking	Maienschein et al. (1999)
Secretogranin II	Stored within dense-core granules	Calegari et al. (1999)
Vacuolar H(+)ATPase	Vesicular proton gradient	Pasti et al. (2001)
VGLUT1/2	Vesicular glutamate transporters	Bezzi et al. (2004)
VGLUT3	Vesicular glutamate transporters	Fremeau et al. (2002)

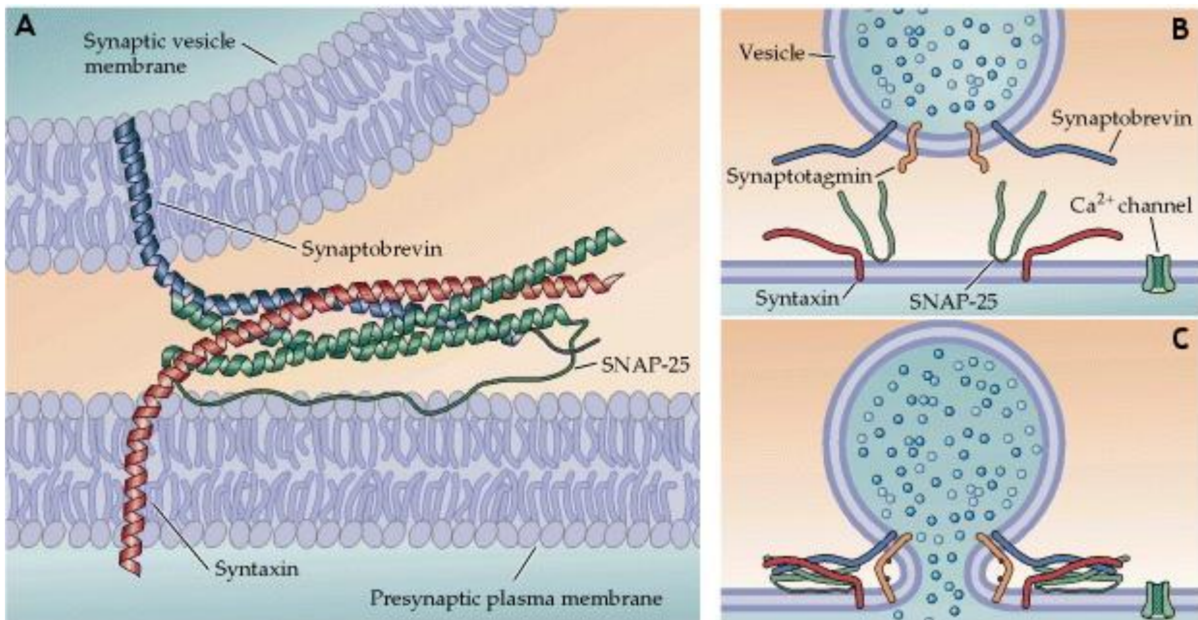

Fig. 10: Molecular mechanisms of neurotransmitter release. (A) Structure of the SNARE complex. The vesicular SNARE, synaptobrevin (blue), forms a helical complex with the plasma membrane SNAREs syntaxin (red) and SNAP-25 (green). (B) A model for Ca^{2+}-triggered vesicle fusion. SNARE proteins on the synaptic vesicle and plasma membranes form a complex (as in A) that brings together the two membranes. Ca^{2+} then binds to synaptotagmin on the vesicle membrane, causing the cytoplasmic region of this protein to insert into the plasma membrane and catalyze membrane fusion.

Table 2: Overview of metabotropic glutamate receptors

Family	**Receptors** [132]	**Function**	**Synapse site**
Group I	mGluR1 and 5	Increase [133,134] NMDA receptor activity	mainly postsynaptic[135]
Group II	mGluR2 and 3	Decrease[136] NMDA receptor activity	mainly presynaptic[135]
Group III	mGluR4, 6, 7 and 8	Decrease[136] NMDA receptor activity	mainly presynaptic[135]

Glutamate

Glutamate is the most abundant excitatory neurotransmitter in the vertebrate central nervous system. In astrocytes, glutamate is synthesized *de novo* as a by-product of the citric acid cycle, because of the conversion of α-ketoglutarate by the enzyme pyruvate carboxylase[126] via transamination of another amino acid, such as aspartate[127]. After synthesis, glutamate is packed in secretory vesicles, attached to the cell membrane by SNARE proteins, prepared for release into the synaptic cleft. Release of glutamate is triggered by high levels of calcium ions in the cytosol, which can happen through various pathways, as described previously.

Most notably is the activation of metabotropic glutamate receptors (mGluR) subtype 1 and 5 by extracellular glutamate, which causes a release of glutamate through activation of PLC and IP_3[128]. This means that when an astrocyte releases glutamate, positive feedback on glutamate release will occur by triggering its own release[129]. Negative feedback will occur shortly after, since the density of astroglial glutamate uptake transporters, or excitatory amino acid transporter 2 (EAAT2), will be rapidly upregulated in a CA-dependent manner during exo/endocytosis[130].

Non-vesicular release seems to be possible when the astroglial P2X7 receptors are activated by ATP or a purinergic ligand derived from ATP. Large ion channels are formed through which glutamate can be released[131]. Secretory vesicles play no role in this kind of exocytosis, and the function of this kind of release is not yet understood.

The effect of glutamate from astroglial or neural origin on neurons is quite similar. Glutamate is the main excitatory neurotransmitter in the CNS, it has effects on LTP and depolarization. Ionotropic glutamate receptors form non-specific cation channels which allow the influx of K^+, Na^+ and CA^{2+} in response to glutamate binding and cause excitatory post-synaptic currents or eventual depolarizations. The ionotropic glutamate receptors are AMPA receptor, kainate receptor and NMDA receptor of which the latter needs depolarization to activate. The metabotropic glutamate receptors act on G-protein coupled receptors by mediating NMDA activity and are described in **Table 2.** References:[132, 133, 134, 135, 136]. The big difference between astroglial glutamate and neural glutamate is the amplitude and duration in which it is released. There is evidence that instead of a sudden and large release of glutamate, similar to neurotransmitter release of a neuron, astrocytes release glutamate in a slow, continuous manner[104]. These observations would imply a great effect of astrocytes on augmenting long-term potentiation by causing a continuous increase of the post-synaptic membrane potential. On the other hand, if astrocytes release slow glutamate currents, these slow currents may induce long-term depression by a continuous moderate Ca^{2+} influx through AMPA receptors, with dephosphorilation of CaMKII and following AMPAR downregulation as result.

D-Serine

D-serine is an endogenous co-activator for the *N-methyl D-aspartate (*NMDA) receptor and is released by astrocytes. It is synthesized by the conversion product of the isomer L-serine by the enzyme serine racemase, an enzyme produced solely by astrocytes[137]. After production it is packaged into secretory vesicles, downstream of the golgi system, but the transporter for this process has not been determined yet[138].

By activation of metabotropic glutamate receptors (mGluRs) the astroglia release D-serine, both *in vivo* as *in vitro*, by a SNARE- and Ca_2^+-dependent vesicle release[139]. When released, D-serine binds to the glycine binding site of the NMDA receptor where it acts as a co-activator alongside glutamate. Interestingly, the binding of D-serine to the NMDA receptor (NMDA-R) is stronger than glycine, what seems to play a negligible role at the NMDA-R[140]. For the activation of the NMDA receptor depolarization of the postsynaptic neuron is needed to void the Mg^{2+}-plug clogging the NMDA-R and inhibiting its activation. When glutamate, D-serine and depolarization meet, the NMDA receptor opens its calcium channel with a large Ca^{2+} influx as result[141]. Since D-serine is required to activate the NMDA receptor, it is an important factor in long-term potentiation and thus plasticity, as described earlier in this paper.

An elegant example of the role of astrocyte-derived D-serine mediated plasticity is in an experiment by Panatier and others in 2006 who examined the astroglial ensheatment of neurons in the supraoptic nucleus (SON) in female rats. In virgin rats, a high degree of astroglial coverage of the synapses caused higher levels of D-serine to reach the NMDA receptors causing LTP. When the rats start to lactate the astrocytes retreat, whereby lower concentrations of D-serine reach the NMDA-R, which has LTD as consequence[142]. Although a recent study has shown the release of D-serine from neurons as well[143], I would conclude that astroglial-derived D-serine appears to be an important part of synaptic plasticity.

ATP

Adenosine-5'-triphosphate (ATP) is produced via glycolysis and oxidative phosphorylation. Because of the well-known function of intracellular ATP as an energy source for a broad variety of processes, the function for ATP as transmitter has not been recognized for long. At first, ATP was discovered as a co-transmitter in (para)sympathetic neurons by G. Burnstock[144], while later it has been recognized as a solitary or co-transmitter in both the peripheral as central nervous system. It is now well established that ATP both acts as a fast excitatory neurotransmitter and has potent long-term (trophic) roles in cell proliferation, growth and development, and in disease and cytotoxicity[145]. ATP release also plays a role in calcium waves, as described previously.

ATP is packed into secretory vesicles by the Cl^--dependent VNUT transporter, which is highly expressed in astrocytes. While concentrations may differ for each vesicle, ATP can be co-stored and co-released with other transmitters like glutamate [purinergic signaling overview]. Like the other gliotransmitters, ATP is released by secretory vesicles in a SNARE and Ca^{2+}-dependent manner[146], but also through other pathways. Similar to glutamate, ATP vesicle release is also activated by the substance itself. Activation of the P2Y1 or P2Y2 metabotropic receptor cause ATP release[68], but also activation of the ionotropic P2X7 receptor causes ATP release through the formation of a large ion pore in a non-vesicular manner[147]. Pannexin (Panx1) membrane channels, which are responsive to increases in intracellular CA concentrations and mechanical stretch, have been proposed to release ATP as well[148].

When released into the synaptic cleft, ATP may be broken down into ADP, AMP or adenosine, by proteins in need for energy or other mechanisms. It comes as no surprise that the receptors for ATP react to the other purines as well. The purinergic receptors might be the most abundant receptors in mammalian tissues[149]. The P1 adenosine receptor acts on G-protein coupled receptors raising or lowering cyclic AMP (cAMP) production, depending on the subtype. The other two types of purinergic receptors know nowadays are the metabotropic P2Y and the ionotropic P2X receptor, with many varieties but a similar function: Increase of the intracellular Ca2+ concentration. Activation of these P2X receptors on several types of CNS neurons, like in the somatosensory cortex, will create an exceptionally high Ca2+ influx, which is comparable to the calcium permeability of the NMDA receptor. In contrast to NMDA receptors, P2X receptors can be activated at resting membrane potentials, suggesting an important role for P2X receptors in calcium signaling in the CNS. Although the average purinergic EPSC has a smaller amplitude than a NMDAR mediated EPSC, in a single synapse the effect may be comparable[150].

It comes as no surprise that purinergic transmission with such strong effects on neural and astroglial calcium concentrations is linked with synaptic plasticity. However, due to the lack of a well understood mechanism, the evidence to whether ATP mediates LTP or LTD remains unclear. For example, in the hippocampus inhibition of P2X transmission has proven to facilitate LTP[151]. On the other hand, knocking out the $P2X_4R$ in mice[152], or antagonism of the P2X in the hippocampus inhibits the formation of LTP[153]. Since purinergic (glio)transmission is such a complex system, further research on this topic is needed to fully understand its mechanisms and implications.

ATP plays a key role in chronic or neuropathic pain[154], due to neural hypersensitivity and plasticity gone awry[155]. Astrocytes have been linked with this problem[156], but at the moment only in the spinal cord[157]. T Microglia, however, seem to play a big role in chronic pain and pain-associated plasticity[158], which is an interesting subject for a thesis about ‘the role of pain and microglia on plasticity’, since more and more evidence about this subject appears. For a review on purinergic signaling and pain, read the review of ref. #159.

Conclusions & Discussion

In summary, astrocytes are able to receive neural signals, communicate with other astroglia and send signals to neurons. Meanwhile, astrocytes are highly motile, release factors that strengthen synapses and permit neurons to survive and function properly. If we then add the evidence that astrocytes release the transmitters needed for successful LTP and absence of astroglia leads to LTD, drawing conclusions seems pretty straightforward. Given the current evidence, I can only conclude that astrocytes are an important part of synaptic plasticity.

However, everything that has been described in this paper is immature information, being no more than 15 years old. The research leading to a brain controlled by astrocytes has not yet reached maturation, but when the time is right, it will be a beautiful paradigm shift. Too much information at this point is uncertain or disputed, while new information emerges every week. When new information takes terrain, old information recedes and is looked upon as old stories, good for a chuckle. If one would tell anyone outside neuroscience that astrocytes are involved in brain communication, most people would think he was raving mad. However, when the roles of astrocytes become more known, a lot will change about what we know of the brain. At this point in time only the effects of one astrocyte and a few synapses can be interpreted. Hoping to see the bigger picture of an intercellular calcium wave of 50 astrocytes, each controlling about 100.000 synapses will drive any sane person mad. One day, the mechanisms driving the brain will be unveiled, but until that day the development of neuroscience will be a fascinating one.

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