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Running title

Q&A on reactive astrocytes

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Abstract

Astrocytes are key cellular partners for neurons in the central nervous system. Astrocytes react to virtually all types of pathological alterations in brain homeostasis by significant morphological and molecular changes. This response was classically viewed as stereotypical and is called astrogliosis or astrocyte reactivity. It was long considered as a non-specific, secondary reaction to pathological conditions, offering no clues on disease-causing mechanisms and with little therapeutic value.

However, many studies over the last thirty years have underlined the crucial and active roles played by astrocytes in physiology, ranging from metabolic support, synapse maturation and pruning to fine regulation of synaptic transmission. This prompted researchers to explore how these new astrocyte functions were changed in disease, and they reported alterations in many of them (sometimes beneficial, mostly deleterious). More recently, cell-specific transcriptomics revealed that astrocytes undergo massive changes in gene expression when they become reactive. This observation further stressed that reactive astrocytes may be very different from normal, non-reactive astrocytes and could influence disease outcomes. To make the picture even more complex, both normal and reactive astrocytes were shown to be molecularly and functionally heterogeneous. Very little is known about the specific roles that each subtype of reactive astrocytes may play in different disease contexts.

In this review, we have interrogated researchers in the field to identify and discuss points of consensus and controversies about reactive astrocytes, starting with their very name. We then present the emerging knowledge on these cells and future challenges in this field.

Keywords

Reactive astrocytes, brain diseases, neuron-glia interactions, astrogliosis, animal models

Main Points

- Astrocytes react to brain homeostasis alteration by morphological and molecular changes
- This response is complex, heterogeneous and subject to controversies
- New tools, models and concepts will help better understand this widespread reaction

1. Introduction

Astrocytes have key functions in the brain (Verkhratsky & Nedergaard, 2018). New discoveries are made regularly on their active roles in ion homeostasis, vascular coupling, synaptic plasticity, circuit building, synapse turnover, waste clearance or higher functions like sleep-wake cycle, food intake or memory (Verkhratsky & Nedergaard, 2018). One peculiar feature of these glial cells, first observed by 19th century anatomopathologists is that they look different in the diseased brain [**Fig. 1**, (P. Chaslin, 1891; Ramón y Cajal)]. The original observations reported morphological and histological changes, with increased fibrillary structures and more visible cellular elements in scarring brain tissue from patients [**Fig. 1**, see also (Liddelow & Barres, 2017)]. Such changes in astrocyte appearance are observed in the brain of patients suffering from a broad range of brain pathologies (*e.g.* epilepsy, neurodegenerative disease, brain cancer, ischemia, infection, axotomy, invasive injury, toxin exposure), as well as in animal models thereof (**Fig. 2,** and references in legend). *Astrocyte reactivity, Astrogliosis, Astrocyte activation, Reactive gliosis* or *Astrocytosis* are among the terms used to describe such molecular, morphological and functional changes in astrocytes. Even if definitions of astrocyte reactivity were proposed by Prof. M. Sofroniew (Sofroniew, 2009, 2014a; Sofroniew & Vinters, 2010) and others (Pekny et al., 2016), a review of the literature shows that the use and meaning of these different terms are quite broad and inconsistent. Indeed, we surveyed ~40 researchers in the field (see complete list in **Supplemental Table 1**), and found that they have quite different visions of what reactive astrocytes are, or are not (**Table 1a**) and they do not agree on the nomenclature (**Table 1b**).

Beyond the discrepant terminology, some common features of reactive astrocytes emerge from this survey. A broad definition could be summarized as "astrocytes sense and respond to an abnormal situation in the brain. They change at the morphological, biochemical, transcriptional and functional levels. These changes are maintained while the pathological stimulus is present but some aspects may resolve. Reactive astrocytes are heterogeneous and may have various effects on disease progression". Indeed, reactive astrocyte heterogeneity is underlined by most researchers and contested by none of them (**Table 1a**). This opens new areas of research to understand its origin and consequences (**Section 5**).

Other points that remain controversial will be covered later in this review: Are glial fibrillary acid protein (GFAP) and morphological changes appropriate markers of reactive astrocytes (**Section 4**)? Can we identify better markers (**Section 6**)? Do reactive astrocytes do good or bad things in disease (**Section 8**)? Do they die (**Section 9**)? Should aging astrocytes be considered as reactive (**Section 12**)? Other points were not necessarily raised by surveyed researchers, but are still actively studied: What are the exact triggers and downstream signaling pathways controlling this response (see **Section 7**)? Where do reactive astrocytes come from and what do they become (see **Section 10**)? How do reactive astrocytes interact with other cell types involved in innate and adaptive immunity (see **Section 11**)? Addressing all these questions will help provide a refined vision of this complex brain response.

The aim of this review is not to provide an extensive overview of the now abundant literature on reactive astrocytes, but instead, discuss emerging research questions and unresolved issues on this topic.

2. How to best name them?

But first, it is important to name our research subject and define what we are talking about. Our survey of researchers in the field of astrocytes, showed that even three broadly-used terms had different inferred meanings (**Table 1b**). Many surveyed PIs agreed that the very definition of astrocyte reactivity was ambiguous and that a clear nomenclature would benefit the field.

The heterogeneity of this response and the variety of "reactive states" (see **Section 5**), call for a broad and inclusive definition that can further be refined in each context. Indeed, all recognized that drastic changes happen to astrocytes in pathological conditions, with some core and some disease-specific alterations (**Table 1a, Supplemental Text**). We thus need words to describe this phenomenon. What are the most appropriate ones? There was some agreement that *astrocyte activation* is misleading because it can apply to physiological activation like transient Ca^{2+} signaling in response to normal neuronal activity (see **Section 4-Timescale**). It is thus better to avoid this term. Another point of partial convergence was that *astrogliosis* implied something different (*e.g.*

stronger or irreversible; with scarring, proliferation or immune infiltration), which definitely does not apply to all disease conditions (**Table 1b**). We are therefore left with the term *astrocyte reactivity*. The problem with this expression, as stressed by several PIs, is that it implies an "ability" to become (See **Table 1a**). As discussed by Dr. A. Serrano-Pozo, *reaction* would be more correct to define the final state of astrocytes (see **Supplemental Text**). We thus propose to avoid the use of *astrocyte activation* and *astrogliosis* and instead use *reactive astrocytes* or *astrocyte reaction* and add *to stroke/epilepsy/Alzheimer disease* to further define it. Expressions like *scar-forming astrocytes*, *proliferating reactive astrocytes, phospho-STAT3⁺ reactive astrocytes* are also very useful to functionally or molecularly define these cells (see details in **Section 5**). We will use this nomenclature in our review, but a more widespread agreement on terminology would be beneficial to the astrocyte community.

3. Why bother with reactive astrocytes?

There are more than 17,500 articles recovered in PubMed with the query "Reactive astrocyte" OR "Astrocyte reactivity" OR "Astrogliosis", with more than 400 articles published each year since 2000. Why does such a broad and somehow ill-defined concept trigger this significant interest? Indeed, if astrocytes become reactive in response to something already going wrong, why should we care? It may already be too late, the initial pathological event may have started well before and triggered an irreversible disease cascade.

It is striking that astrocyte reaction is such a widespread response, reported in virtually all brain diseases, brain regions, and multiple species including invertebrates, like *Drosophila*, many mammals and Humans [(Kremer, Jung, Batelli, Rubin, & Gaul, 2017; Sofroniew & Vinters, 2010), see **Fig. 2**]. It is thus very important to better understand what it means for an astrocyte to engage in this phenotype switch and how it can influence surrounding cells.

Astrocyte reaction is by definition a secondary event; yet, it may still impact disease progression, which lasts for months or years in the case of tumor, neurodegenerative disease or epilepsy. More importantly, astrocytes are reactive at the clinically visible stages of the disease, when patients seek diagnosis and treatment. Turning reactive astrocytes into beneficial partners for vulnerable neurons exposed to a chronic disease or to the long-term consequences of an acute injury, would be a valuable therapeutic strategy.

In addition, as astrocytes react to altered homeostasis in the brain, they are endogenous biomarkers for brain diseases. Development of non-invasive imaging techniques, like magnetic resonance imaging (MRI) or positron emission tomography (PET) to visualize when and where astrocytes become reactive would help disease diagnostic. Available imaging methods are not quite specific for reactive astrocytes, they rather detect neuroinflammation as a whole (i.e. reactive glial cells, sometimes infiltration of peripheral immune cells) or associated changes, for example in brain metabolism (Aiello et al., 2018; Carrillo-de Sauvage et al., 2015; Lavisse et al., 2012). Indeed, astrocytes may significantly contribute to imaging signals, such as those measured with blood oxygenation level-dependent (BOLD) functional MRI or [^{18}F]-fluorodeoxyglucose uptake by PET, and therefore their reactive state may impact these measurements (Carter et al., 2019; Mishra, 2017). Refined approaches that are more selective for reactive astrocytes are being developed (Carter et al., 2019; Ligneul et al., 2019; Rodriguez-Vieitez et al., 2016; Scholl et al., 2015). Such improvements will be facilitated by basic studies that establish the molecular and functional profile of reactive astrocytes (see **Section 6**) but also of their microglia or neuronal neighbors, allowing the identification of new and specific astrocyte targets.

Last, some astrocyte proteins like GFAP or their break-down products may also end up in the cerebrospinal fluid of patients subject to traumatic brain injury [TBI, (Halford et al., 2017)], but also in more progressive diseases like Creutzfeldt-Jakob disease or Alzheimer disease (AD) [for review, see (Carter et al., 2019; Perez-Nievas & Serrano-Pozo, 2018)].

Therefore, defining how astrocytes change during disease may *in fine* lead to new biomarkers, better diagnosis tools, and even original therapeutic strategies, which are long-sought goals for many diseases.

4. What are the defining features of reactive astrocytes?

Several common features are observed in very different cases of brain injuries and diseases. They can be used to define the core hallmarks of reactive astrocytes.

Morphological changes. In pathological conditions, astrocytes display morphological changes, including hypertrophy of soma and main processes, but not only (see **Fig. 2**). This was already noted by earlier pathologists using impregnation techniques to achieve sparse labeling of astrocytes (**Fig. 1**). Diolistic labeling, dye-filling or expression of a cytosolic or membrane-tagged fluorescent protein reveal the complex and highly ramified astrocyte morphology. These methods evidence subtle alterations in astrocyte morphology that could be missed by cytoskeleton labeling with GFAP antibodies (See **Fig. 2B, D, E, H, I, J**). They show process polarization towards a lesion [(Bardehle et al., 2013), **Fig. 2H**] or ramification changes while the overall 3D domain covered by a single astrocyte is not massively disrupted [(Wilhelmsson et al., 2006), **Fig. 2E**]. In more severe diseases like epilepsy, astrocytes may also retract their fine peripheral processes, become asymmetric and overlap more with their neighbors [(Oberheim et al., 2008; Sun & Jakobs, 2012), **Fig. 2J**]. An extreme case is the glial scar caused by mechanical lesions of brain or spinal parenchyma (**Fig. 2F**), or severe focal lesions like tumors (**Fig. 2R**). Astrocytes become elongated and assemble to form a compact and permanent scar. Other cell types, such as fibroblasts, oligodendrocyte progenitor cells (OPC), ependymal cells or pericytes, may also contribute to this scar (Adams & Gallo, 2018; Sabelstrom, Stenudd, & Frisen, 2014). This question and the overall effect of the glial scar on axonal regrowth are still debated (Anderson et al., 2016; Silver, 2016). Readers are referred to excellent reviews on the topic (Adams & Gallo, 2018; Sofroniew, 2018).

Molecular changes. A defining feature of reactive astrocytes is their overexpression of intermediate filament proteins like GFAP or vimentin. The *"increased number and size of fibrils in neuroglia of sclerotic brain tissue in epileptic patients"* was noted by anatomopathologists already at the end of the 19th century [see **Fig. 1,** (P. Chaslin, 1891)]. Electron microscopy also evidences large bundles of filaments in reactive astrocytes (**Fig. 2M'**). In AD brains, GFAP accumulates to the extent that it forms protein aggregates resembling Rosenthal fibers in reactive astrocytes (Wegiel & Wisniewski, 1994b). The surveyed researchers broadly acknowledged the validity of GFAP as a marker for reactive astrocytes (**Table 1a**), but several of them also underlined its limits (see further discussion in **Section 6**).

Owing to refined methods to isolate astrocytes and investigate their transcriptome at the genome-wide level, recent studies revealed that astrocyte reaction involves massive transcriptional changes that go well beyond *Gfap* induction. Hundreds of genes are either up- or down-regulated in astrocytes in AD models and patients (Ceyzériat et al., 2018; Orre, Kamphuis, Osborn, Jansen, et al., 2014; Sekar et al., 2015), in a mouse model of hyperammonia (Lichter-Konecki, Mangin, Gordish-Dressman, Hoffman, & Gallo, 2008), or multiple sclerosis (MS) (Itoh et al., 2018), following spinal cord injury [SCI, (Anderson et al., 2016)], cortical stab wound injury [SWI, (Sirko et al., 2015)], middle cerebral artery occlusion (MCAO) or lipopolysaccharide (LPS) injection (Zamanian et al., 2012). Several genes induced in reactive astrocytes both in the LPS and MCAO models were identified. Among them, *Serpina3n* and *Lcn2* were further validated as strongly, but transiently induced following LPS injection or MCAO (Zamanian et al., 2012). They are also induced in other disease models (Itoh et al., 2018; Suk, 2016; Switonski, Szlachcic, Krzyzosiak, & Figiel, 2015). Change in transcriptome is a conserved feature of astrocyte reaction, similarly observed in *Drosophila* glia (Lu et al., 2017).

Interestingly, genes that are down-regulated in reactive astrocytes may also hold the key to understanding the roles of these cells in disease. Some genes associated with important astrocyte functions like the potassium channel KIR4.1 [*Kcnj10,* (Nwaobi, Cuddapah, Patterson, Randolph, & Olsen, 2016)], the glutamate transporter GLT1 (*Slc1a2*) or glutamine synthase GS [*Glul,* (Sheldon & Robinson, 2007)], are repeatedly reported as down-regulated in disease. Likewise, reduced expression of several homeostatic astrocyte genes is reported in mouse models of SWI (Sirko et al., 2015) and TBI (Shandra et al., 2019) (see also **Section 10**), but there is no established list of genes down-regulated in reactive astrocytes across multiple diseases. Overall, the significant phenotypic alterations observed in reactive astrocytes involve large-scale transcriptome modifications and functional changes (see **Section 8**).

Migration & proliferation. The very first observers of astrocytes noticed increased numbers of nuclei in sclerotic tissue of epileptic brains, and discussed the proliferative capacity of neuroglia [**Fig. 1**, (P. Chaslin, 1891; Ramón y Cajal)]. But more recent studies based on two-photon imaging of astrocyte reaction, fate mapping and bromodeoxyuridine (BrdU) labeling in animal models, challenged this dogma [(Bardehle et al., 2013; Buffo et al., 2008), **Fig. 2H**]. Immunostaining with Ki67 or Proliferating Cell Nuclear Antigen in patients confirmed that only a small percentage of reactive astrocytes undergoes proliferation (Perez-Nievas & Serrano-Pozo, 2018). These proliferative astrocytes appear to require a stimulus from outside the central nervous system (CNS): they are in direct contact with the lesion in SCI [(Wanner et al., 2013), **Fig. 2F**], are exposed to blood-borne substrates following SWI (Sirko et al., 2013), or their cell bodies are in apposition to blood vessels (Bardehle et al., 2013). Notably, proliferative astrocytes perform only one or two rounds of division (Bardehle et al., 2013; Sirko et al., 2013). Two-photon microscopy also showed that reactive astrocytes do not migrate like microglia or macrophages do after a focal mechanical injury (Bardehle et al., 2013; Nimmerjahn, Kirchhoff, & Helmchen, 2005). Similarly, in the spinal cord, astrocytes remain in their allocated regional domain and do not migrate to a nearby SWI (Tsai et al., 2012).

Timescale. Increased GFAP and nestin protein expression is detected as soon as 90 min after mouse euthanasia and preparation of acute slices, showing that molecular changes may occur rapidly (and even *ex vivo*) (Takano et al., 2014). In addition, In different striatal injury models caused by acute neurotoxin injection, *Gfap* mRNA levels are induced 6 h later, while GFAP protein levels increase significantly only after 12-72 h, depending on the model (O'Callaghan, Kelly, VanGilder, Sofroniew, & Miller, 2014). Astrocyte reaction can be viewed as a change of state or a conversion (**Table 1a**). By becoming reactive, astrocytes undergo a set of morphological, transcriptional and functional changes that transform them into different cells, with acquired, lost or altered properties and functions (see **Section 8**). This is different from a rapid stimulation by neurotransmitters for example, which will produce transient movements of perisynaptic processes (Bernardinelli et al., 2014), Ca^{2+} signals or gliotransmitter release (Araque et al., 2014). This physiological response (which is better qualified as activation, see **Table 1b** and **Section 2**), will not necessarily be long-lasting or associated with large scale transcriptional changes that shift astrocyte phenotype. On the contrary, reactive astrocytes may persist over months and even years in chronic brain diseases, although they may evolve overtime.

Reversibility. Astrocyte reaction is reversible and may resolve. Manipulation of specific signaling cascades *in vitro* and *in vivo* can normalize the transcriptome of astrocytes. Whether such "de-activated" astrocytes are truly normal or are in a different state remains to be fully explored. Indeed, it is known that a given stimulus has a different effect on astrocytes, when they were previously exposed to a first injury, a process known as priming (Hennessy, Griffin, & Cunningham, 2015). A very elegant study recently showed that grafting reactive astrocytes in a normal mouse spinal cord is sufficient to revert them to a non-reactive state (Hara et al., 2017). Conversely (but less surprising), grafting normal astrocytes in an injured environment turns naïve astrocytes into reactive astrocytes based on both morphological and molecular criteria, showing that reactive astrocytes are plastic and regulated by environmental cues. Among the reported signals involved in maintaining a non-reactive state or resolving astrocyte reaction are fibroblast growth factor (Kang et al., 2014), β1 integrin (Hara et al., 2017; Robel et al., 2009), transforming growth factor α (Rothhammer et al., 2018), microbiome-derived tryptophan metabolites (Rothhammer et al., 2016), suppressor of cytokine signaling 3 [SOCS3, (Ceyzériat et al., 2018)] or Sonic Hedgehog [SHH, (Garcia, Petrova, Eng, & Joyner, 2010)). Such signaling molecules offer unique opportunities to tune astrocyte reaction and evaluate the consequences on specific outcomes in different disease models (see **Section 8**).

5. Do all astrocytes react the same way?

As nearly all surveyed researchers noted (**Table 1a**), the global expression "*reactive astrocytes*" falls short of describing the different responses that astrocytes may express in different disease conditions or even in response

to the same stimulus (**Table 2**). As Prof. A. Messing puts it, citing Leo Tolstoy, "*All happy families are alike; each unhappy family is unhappy in its own way*", suggesting that astrocytes react in a specific manner in each pathological situation. This was nicely illustrated by two-photon monitoring of astrocyte responses following focal SWI. All astrocytes proximal to the lesion became hypertrophic but only half of them polarized their processes towards the lesion, the other half remained static and only 10% of them underwent proliferation [(Bardehle et al., 2013), **Fig. 2H**]. Likewise, a lineage tracing method was used to determine whether astrocytes derived from the same developmental clone display similar morphological changes following SWI or experimental autoimmune encephalomyelitis (EAE). In both cases, reactive astrocytes from the same clone tended to behave similarly, indicating that they are controlled by intrinsic cues established during development. However, some astrocytes reacted differently than other cells in the same clone, showing that environmental signals further diversify astrocyte response [(Bribian, Perez-Cerda, Matute, & Lopez-Mascaraque, 2018; Martin-Lopez, Garcia-Marques, Nunez-Llaves, & Lopez-Mascaraque, 2013), **Fig. 2B**].

The heterogeneity of reactive astrocytes is also evidenced by spatial gradients in the intensity of response, which culminates at the core of ischemia injury, SCI, mechanical lesion or epileptic focus (see **Fig. 2A** for example). This is defined as topographical heterogeneity (Anderson, Ao, & Sofroniew, 2014) and is basically determined by the distance to the injury core. As the concentration of molecular stimuli decreases with distance to the injury core, so does astrocyte reaction, at least based on morphological changes or GFAP induction (see **Table 2**). As Prof. S. Sirko explains it "*as in Newton's third law, the action and the reaction [of astrocytes] are equivalent in magnitude*". But this apparent continuum in the intensity of astrocyte reaction may in fact hide different, discrete reactive states. For example, proliferative reactive astrocytes are only found proximal to the lesion [(Herrmann et al., 2008; Wanner et al., 2013), **Fig. 2F**]. Nearly ten years ago, Prof. M. Sofroniew described three categories of astrocyte reactivity: mild to moderate; severe diffuse and severe with glial scar. It was a noticeable effort to better define this process and recognize its heterogeneity (Sofroniew & Vinters, 2010). This classification is based on measurable criteria (proliferation, process extension, maintenance or loss of exclusive 3D domains) but there is no absolute quantitative categories (*e.g.* to what extent astrocytes need to elongate their processes to fall into the mild or severe category?). This classification may thus be difficult to translate to any disease or model, and does not take advantage of objective molecular markers. Indeed, it was recently shown that reactive astrocytes that form the glial scar and lose their 3D domain have a different molecular profile than hypertrophic reactive astrocytes located farther away. "Scar-forming" astrocytes express specific transcripts such as chondroitin sulfate proteoglycans *(e.g. Acan, Pcan)* and N-Cadherin *(Cdh2),* while hypertrophic reactive astrocytes express several members of the β-catenin pathway (*e.g. Ctnnb1, Plaur*) (Hara et al., 2017). Interestingly, both types of reactive astrocytes overexpress *Gfap* and *Vim* compared to normal astrocytes (Hara et al., 2017). In addition, scar-forming astrocytes proliferate more than more distant hypertrophic reactive astrocytes [see **Section 6, Table 2,** (Wanner et al., 2013)]. Overall, these data suggest that the scar-forming astrocytes are a specific reactive astrocyte subtype and not a mere exacerbation of hypertrophic reactive astrocytes.

Other sources of heterogeneity have been described, such as regional differences, even more than 20 years ago (**Table 2**). Astrocytes from different CNS regions exposed to the same toxic stimulus (Amyloid β) respond differently *in vitro* (Hoke, Canning, Malemud, & Silver, 1994), showing that astrocyte heterogeneity is at least partially intrinsic, as it is maintained in a dish. Likewise, astrocytes from gray and white matter react differently to SWI (Mattugini et al., 2018). This could be due to pre-existing differences between astrocytes from distinct brain regions (Boisvert, Erikson, Shokhirev, & Allen, 2018; Chai et al., 2017; Itoh et al., 2018; John Lin et al., 2017; Lanjakornsiripan et al., 2018; Morel et al., 2017; Torigoe, Yamauchi, Zhu, Kobayashi, & Murakami, 2015). Another demonstration of different molecular classes of reactive astrocytes came from the Barres laboratory. They reported that the molecular changes induced in astrocytes by LPS injection or MCAO only partially overlap (Zamanian et al., 2012). This led to the description of A1 and A2 reactive astrocytes, each characterized by a set of 10-13 genes, in addition to a panel of 10-13 common genes (i.e. pan reactive) including *Gfap, Vimentin* and *Serpina3n* (Liddelow et al., 2017). The molecular profile of LPS-induced A1 reactive astrocytes was replicated quite faithfully by treating immunopanned rodent astrocytes with interleukin 1α (IL1α), complement 1q (C1q) and tumor necrosis factor (TNF) (Liddelow et al., 2017). A detailed functional characterization of A1 reactive astrocytes showed that they release factors that are toxic to neurons and oligodendrocytes, are less synaptogenic

and have defective phagocytosis. But this dual classification may be quite restrictive to define other potential types of reactive astrocytes existing in the complex world of brain diseases (Cunningham, Dunne, & Lopez-Rodriguez, 2018). Indeed, many intermediate molecular profiles were observed after treatment of immunopanned astrocytes with different molecules, not only A1 or A2 (Liddelow et al., 2017). In a complex *in vivo* multicellular environment with multiple stimuli, reactive astrocyte diversity may even be stronger. Both A1 and A2 genes are induced concomitantly in different models, such as mouse models of AD (Ceyzériat et al., 2018), Tauopathy (Litvinchuk et al., 2018) or ischemia (Liddelow et al., 2017).

Overall, some markers may display graded changes, proportional to the intensity of the initial injury [GFAP, cytokines, (Sofroniew, 2014a)], while others may undergo an all-or-none response, being present only in some forms of reaction [*e.g.* A1 versus A2 reactive astrocytes (Liddelow et al., 2017); scar-forming versus hypertrophic reactive astrocytes (Hara et al., 2017), ephrin type-B receptor 1 (EphB1) versus interleukin (IL)6-induced reactive astrocytes (G. E. Tyzack et al., 2017), **Table 2**].

There are several origins and several manifestations of reactive astrocyte heterogeneity (**Fig. 3, Table 2**). Indeed, as stated by Prof. A. Volterra « *the combination of the intrinsic features of a given astrocytic population, of its microenvironment and the type of insult will concur to produce different types of reactivity and different functional outcomes*». More precisely, astrocyte reaction is determined by core changes (induction of *Gfap* expression, morphological plasticity), combined with disease-specific changes on a preexisting heterogeneous background (**Fig. 3**), resulting in an extreme variety of possible reactive astrocyte subtypes. This leads us to the next question, very central to the field.

6. What are the appropriate markers of reactive astrocytes?

As discussed earlier, GFAP overexpression and morphological changes are the most commonly used of reactive astrocyte markers (Liddelow & Barres, 2017). Both are easy to monitor on different types of samples (see **Fig. 2, Table 2**). *Gfap* is one of the most consistently induced gene in transcriptomic datasets of reactive astrocytes, confirming its usefulness as a reactive marker [(Hol & Pekny, 2015; Liddelow et al., 2017; Orre, Kamphuis, Osborn, Jansen, et al., 2014; Zamanian et al., 2012), see also **Table 2** and **Section 4]**. It is important to note however, that the level of *Gfap* induction can be very different between conditions (see **Table 2** and **Section 5**) or even between cells (Pekny, Wilhelmsson, Tatlisumak, & Pekna, 2019), but GFAP expression globally increases at the population level, in a wide range of brain diseases.

Are GFAP induction and morphological changes enough to identify reactive astrocytes? Several surveyed PIs think that these indexes can be misleading (**Table 1a**). Indeed, additional markers would be helpful with some specific forms of reactive astrocytes that display unconventional morphological alterations or lower GFAP immunoreactivity (see **Section 9**). In addition, they would be useful to define specific types of reactive astrocytes. Genome-wide transcriptional profiling can identify potential common and class-specific genes (Hara et al., 2017; Liddelow et al., 2017). How many markers are needed to define a class? For example, of the ~10 genes forming the A1 and A2 panels, should they all be induced in a given condition to qualify a cell as an A1 reactive astrocyte or 2-3 are enough? In fact, the expression of only a few protein markers (Complement 3, Complement factor b and MX dynamin-like GTPase 1) was tested in the brain of patients and these three genes were not in the original A1 panel [(Liddelow et al., 2017), **Fig. 2T, U**]. When the full panel of A1 genes is tested, not every single gene of the A1 cassette is induced in several diseases, like *in vitro* and *in vivo* models of Parkinson disease (Yun et al., 2018) or AD mouse models (Ceyzériat et al., 2018). In addition, individual reactive astrocytes co-expressing A1 and A2 genes are observed in the MCAO rodent model and in the aging mouse brain (Clarke et al., 2018), suggesting that it may be difficult to define classes of reactive astrocytes with only a few genes.

To improve the panel of reactive astrocyte molecular markers, it would be useful to extend the transcriptomic analysis of reactive astrocytes performed by Zamanian *et al.* in LPS and MCAO models (Zamanian et al., 2012), to many different, genetic and sporadic, acute and chronic, degenerative and inflammatory diseases. It would help define with greater power the core sets of genes systematically induced or down-regulated in reactive astrocytes, and identify disease-specific markers. Of course, such analysis would provide even more insight if performed on human samples. However, *post mortem* delays induce noise and artifacts and the physical isolation of astrocytes

is quite difficult, although it may be possible to gain some insight into astrocyte-specific transcriptomic changes by co-expression analysis on bulk samples (Kelley, Nakao-Inoue, Molofsky, & Oldham, 2018). A method of choice to study cell diversity at the molecular level is single-cell RNAseq (scRNAseq) (Svensson, Vento-Tormo, & Teichmann, 2018) or single-nuclei RNAseq (snRNAseq) (Habib et al., 2017). These methods have gained significant momentum, and several landmark papers have reported brain cell heterogeneity, including glial cells, throughout development or between regions (Macosko et al., 2015; Pollen et al., 2014; Zeisel et al., 2018). There are only few articles on brain diseases, and to date, only on other glial cells like microglia (Hammond et al., 2018; Masuda et al., 2019). Such unsupervised approaches will help define populations of reactive astrocytes with associated gene markers in different conditions (see **Section 13**).

Another option to classify reactive astrocytes is to combine molecular with functional indexes. A good example is the variable capacity of reactive astrocytes to proliferate. As mentioned earlier, only a subset of reactive astrocytes undergo cell division. These proliferative astrocytes may thus be considered as a different class, of particular interest because they may repopulate the damaged brain (see **Section 10**). But other specific functions (either gained, lost or altered, see **Section 8**) may also serve as functional markers of reactive astrocytes, like enhanced phagocytic capacities around plaques, as shown for "disease associated microglia" (Keren-Shaul et al., 2017). For example, only a fraction of reactive astrocytes phagocyte myelin debris in the brain of patients with MS or leukoencephalopathy [(Ponath et al., 2017), **Fig. 2N**]. A classification based on production of γ-amino-butyric acid (GABA) and brain-derived neurotrophic factor (BDNF) by reactive astrocytes was proposed by Prof. C.J. Lee [see **Table 1a,** and **Supplemental text**, (Chun et al., 2018)], since reactive astrocytes produce more GABA in AD (Jo et al., 2014b; Z. Wu, Guo, Gearing, & Chen, 2014). This is a potentially interesting functional classification of reactive astrocytes, but it needs to be further explored, to evaluate whether this is translatable to multiple diseases, animal models and of course patients. For example, contrary to AD models, astrocyte GABA production is reduced in a mouse model of Huntington disease [HD, (Jo et al., 2014b; Wojtowicz, Dvorzhak, Semtner, & Grantyn, 2013)]. Importantly, a good marker needs to be easy to use and compatible with other exploratory techniques like electrophysiology or functional imaging. Therefore, mRNA and protein markers remain favored options.

A problem that may be very difficult to overcome is the dynamic, flexible and context-dependent phenotype of astrocytes. It was nicely discussed for astrocytes in physiology (Poskanzer & Molofsky, 2018), and it may even be exacerbated in disease, when astrocytes express gene markers of other cell types and cell identities become blurrier (see **Section 10**). It may thus be impossible to establish fixed classes and we rather should try to define states, dependent on previous phenotype, activated signaling and environment (see **Fig. 3**). The ambitious Human Cell Atlas project that aims to define all cells in the human body with a range of single-cell approaches will probably provide the astrocyte field with new tools and concepts to better address the challenge of reactive astrocyte classification.

7. What are the molecular cascades triggered in reactive astrocytes?

First, astrocytes sense a pathological signal. This signal can be extracellular (*e.g.* cytokines, purines, aggregated proteins, myelin debris), trans-cellular (*e.g.* transmembrane adhesion molecules like ephrins or integrins), membrane bound [*e.g.* phosphatidyl-serine (Chung et al., 2013)], as well as intracellular (*e.g.* aggregated proteins, nucleic acids from infecting pathogens, ions like Ca^{2+}), [see (Buffo, Rolando, & Ceruti, 2010; Cunningham et al., 2018; Kang & Hebert, 2011; Sofroniew, 2014a) for review]. Interestingly, a recent study reported that astrocytes are also very sensitive to environmental pollutants (Wheeler et al., 2019). To sense all these molecular triggers, astrocytes are equipped with a wide range of membrane or intracellular receptors, including G Protein-coupled receptors (GPCR, like metabotropic glutamate or P2Y purinergic receptors), ionotropic receptors (*e.g.* P2X purinergic receptors), multimeric cytokine receptors (*e.g.* IL6 family receptors), Toll like receptors [TLR, although their expression by astrocytes is quite disputed (Cunningham et al., 2018)] or, tyrosine kinase receptors (*e.g.* Epidermal growth factor receptor), [see (Kang & Hebert, 2011; Verkhratsky & Nedergaard, 2018) for review]. It is important to note however that there is rarely a direct and formal demonstration that each of these stimuli can trigger a full reactive program (i.e. resulting in morphological as

well as complex transcriptional changes), without the involvement of other cell types like microglia. Indeed, this is typically tested by applying high doses of these compounds *in vitro* or through injection or overexpression *in vivo*. It is therefore difficult to know whether they activate astrocytes directly or through microglial cells (see **Section 11**). Interestingly, astrocytes are also mechano-sensitive. They can detect changes in their environment mechanical properties, discriminate soft from stiff material and adjust by changing their own stiffness (Moeendarbary et al., 2017; Moshayedi et al., 2014).

After stimulus sensing, a step of signal transduction takes place and converge to the nucleus. This can occur through direct shuffling of activated down-stream transcription factors to the nucleus after phosphorylation, dephosphorylation or release from inhibitors. Signal transducer and activator of transcription 3 (STAT3), nuclear factor κB (NF-κB) and nuclear factor of activated T-cells (NFAT), the downstream effectors of the Janus kinase (JAK)-STAT3, NF-κB and calcineurin-NFAT pathways respectively, are regulated by such a mechanism (Ceyzériat, Abjean, Carrillo-de Sauvage, Ben Haim, & Escartin, 2016; Sompol & Norris, 2018; Q. Zhang, Lenardo, & Baltimore, 2017). Many signaling cascades are associated with astrocyte reaction (L. Ben Haim, Carrillo-de Sauvage, Ceyzeriat, & Escartin, 2015; Buffo et al., 2010; Kang & Hebert, 2011), but the STAT3 pathway seems to play a prominent role in different disease conditions, acting as a master regulator of reactive astrocytes [(L. Ben Haim, Ceyzeriat, et al., 2015; Ceyzériat et al., 2016; Herrmann et al., 2008), **Fig. 2O**]. Ca^{2+} signaling may also be involved in astrocyte reaction. Ca^{2+} can activate or inhibit many downstream signaling intermediates like the phosphatase calcineurin (Sompol & Norris, 2018) or the transcriptional repressor Pumilio 2 (Kanemaru et al., 2013), which will activate other downstream pathways like the NFAT or N-cadherin pathways respectively and trigger important transcriptional changes.

Activation of specific transcription factors will induce expression of target genes such as *Gfap or* cytokines but also of transcription factors or retro-inhibitors of other pathways (*e.g.* SOCS3 for the JAK-STAT3 pathway or IκB for the NF-κB pathway), which will further shape the transcriptome of reactive astrocytes. This step may involve successive waves of transcriptional regulation, starting with induction of immediate early genes (Jenab & Quinones-Jenab, 2002; Priller, Reddington, Haas, & Kreutzberg, 1998; Y. E. Wu, Pan, Zuo, Li, & Hong, 2017), followed by subsequent waves of gene induction. The transcription of some genes may be repressed. Indeed, the number of down-regulated genes may even be higher than those over-expressed, as observed AD mouse astrocytes (Orre, Kamphuis, Osborn, Jansen, et al., 2014). Importantly, the precise time course of transcriptional induction is not very well known. It was established for specific transcripts or proteins (mostly GFAP and cytokines) in mice exposed to neurotoxins (O'Callaghan et al., 2014) or LPS (Biesmans et al., 2015; Norden, Trojanowski, Villanueva, Navarro, & Godbout, 2016). Transcriptional profiling of astrocytes was performed in a mouse model of epilepsy, in two brains regions and three time points following pilocarpine injection (Clasadonte et al., 2016). This extensive analysis showed subtle differences between disease stages or brain regions (see **Table 2**). Such genome-wide, longitudinal analysis of relevant *in vivo* models of other brain diseases would provide key insight into the temporal regulation of astrocyte reaction.

Some changes observed in reactive astrocytes do not require transcriptional induction, such as morphological plasticity. The molecular mechanisms underlying astrocyte morphological changes and process motility are mostly studied *in vitro*, where these cells are grown in 2D and usually have a much simpler morphology (sometimes even no processes) [see (Schiweck, Eickholt, & Murk, 2018), for review]. But *in vivo*, reactive astrocytes do not migrate [(Bardehle et al., 2013), **Section 2**] and show little motility of main processes compared to microglia (Nimmerjahn et al., 2005), calling for *in vivo* validation of the signaling described *in vitro*. For example, knockout of the Rho GTPase cdc42 reduces astrocyte polarization *in vitro* in the scratch wound assay, while it exacerbates it after SWI *in vivo* (Robel, Bardehle, Lepier, Brakebusch, & Gotz, 2011). As mentioned earlier (**Section 3**), reactive astrocytes not only present hypertrophy, but also subtle morphological changes. For example, reactive astrocytes retract their processes following stimulation of Slit-Robo signaling by neuroblasts migrating from the subventricular zone after stroke. This cdc42-dependent remodeling of astrocyte cytoskeleton facilitates neuroblast migration towards lesion sites (Kaneko et al., 2018).

Less is known about mechanisms of epigenetic regulation in reactive astrocytes in disease. Based on what happens during astrocyte development, it is suspected that epigenetic processes (chromatin remodeling through

DNA methylation or histone post-translational modifications, as well as expression of regulatory microRNA) could influence astrocyte conversion into reactive cells (Neal & Richardson, 2018).

The profound transcriptional changes occurring in reactive astrocytes translate into functional alterations that may impact virtually all astrocyte functions.

8. Do reactive astrocytes do good things?

Reactive astrocytes are the usual suspects in many diseases, but they are now considered by many, as beneficial partners for neurons, as illustrated in **Table 1a**. An overview of the literature shows that depending on the strategy employed to modulate reactive astrocytes (*e.g.* pharmacological or genetic approaches, targeting of astrocyte proteins or signaling pathways, ablation of astrocytes), the outcomes vary (L. Ben Haim, Carrillo-de Sauvage, et al., 2015; Cunningham et al., 2018). Of course, the disease studied and its model itself (*e.g.* transgenic versus knock-in, *in vivo* versus *in vitro*, species) will influence how astrocyte reaction will play out. More importantly, some models do not reproduce astrocyte reaction well. For example, most HD mouse models do not display reactive astrocytes, while GFAP up-regulation is robustly detected in the caudate-putamen of HD patients (Faideau et al., 2010; Selkoe, Salazar, Abraham, & Kosik, 1982; Vonsattel et al., 1985). This could be due to low GFAP expression in the mouse striatum compared to other brain regions (Chai et al., 2017), significant species differences (Oberheim et al., 2009; Y. Zhang et al., 2016) or a simple failure of mouse models to recapitulate all HD features.

How do reactive astrocytes functionally impact the diseased brain? Virtually all astrocyte functions are reported to be altered in disease: neurotransmitter uptake (Escartin et al., 2006; Sheldon & Robinson, 2007), gliotransmitter release (Jo et al., 2014a; Z. Wu et al., 2014), metabolic activity (Escartin et al., 2007; Gavillet, Allaman, & Magistretti, 2008; Valenza et al., 2010), ion buffering (Tong et al., 2014), release of cytokines, complement factors or trophic factors (Chou et al., 2008; Lian et al., 2015; Sofroniew, 2014b), phagocytosis (Gomez-Arboledas et al., 2018; Liddelow et al., 2017; Morizawa et al., 2017), production and detoxification of reactive oxygen species [ROS, (Allaman et al., 2010; Cassina et al., 2008; Ye et al., 2015)]. These functions may be enhanced or reduced by the reactive state. For example, reactive astrocytes may display enhanced or reduced connectivity through gap junctions, depending on the disease (Escartin & Rouach, 2013; Pannasch & Rouach, 2013). They may display deficits in glutamate uptake (Sheldon & Robinson, 2007), or show enhanced uptake capacity when reaction is induced by the cytokine ciliary neurotrophic factor (CNTF) (Escartin et al., 2006). Reactive astrocytes may release more GABA in AD mice (Jo et al., 2014b; Z. Wu et al., 2014) or do the opposite in HD mice (Wojtowicz et al., 2013).

It is beyond the scope of this review to list all their described functional changes and contributions to specific diseases. Readers are referred to recent reviews on astrocytes in HD (Khakh et al., 2017), AD (Chun & Lee, 2018; Osborn, Kamphuis, Wadman, & Hol, 2016; Perez-Nievas & Serrano-Pozo, 2018), MS (Wheeler & Quintana, 2019), SCI (Adams & Gallo, 2018; Sofroniew, 2018), TBI (Burda, Bernstein, & Sofroniew, 2016), stroke (Pekny et al., 2019), and epilepsy (Coulter & Steinhauser, 2015; Robel & Sontheimer, 2016). In general, deficits in normal astrocyte functions are reported and the existence of "killer astrocytes" releasing toxic molecule(s) was even proposed in amyotrophic lateral sclerosis [ALS, (Haidet-Phillips et al., 2011; Nagai et al., 2007)] and other neurodegenerative diseases [(Liddelow et al., 2017), see **Section 5**]. But there are also strong evidence for beneficial reactive astrocytes in acute or chronic diseases. For example, over the years, the Sofroniew laboratory has convincingly demonstrated that scar-forming reactive astrocytes demarcate damaged tissue (Bush et al., 1999; Faulkner et al., 2004) and even promote axonal regrowth through the lesion (Anderson et al., 2016). Likewise, STAT3-dependent reactive astrocytes reduce degeneration of axotomized motoneurons in the facial motor nucleus, and promotes synapse maintenance through thrombospondin 1 release (G. E. Tyzack et al., 2014). CNTF-induced reactive astrocytes display enhanced glutamate uptake, promote metabolic resilience and protect neurons against excitotoxicity (Beurrier et al., 2010; Escartin et al., 2006; Escartin et al., 2007). Even A1 neurotoxic reactive astrocytes may have some beneficial roles in specific diseases, for example by killing damaged, dysfunctional or infected neurons (Liddelow et al., 2017).

A way to study common functional features of reactive astrocytes, is to activate (or inhibit) a specific signaling pathway to trigger reactive conversion (see **Section 8**). For example, JAK-STAT3 pathway activation in astrocytes induces many reactive markers and is sufficient to alter synaptic transmission and plasticity in the hippocampus (Ceyzériat et al., 2018). Activation of the NF-κB pathway by expression of a constitutive form of the upstream IκB kinase (IKK) in adult astrocytes also induces a reactive profile and Purkinje cell loss in the cerebellum (Lattke et al., 2017; Oeckl, Lattke, Wirth, Baumann, & Ferger, 2012). Such approaches are useful to isolate functional changes in reactive astrocytes, independently of a specific disease condition. Alternatively, blocking astrocyte reaction may help uncover the net effect of reactive astrocytes in each disease context [see (L. Ben Haim, Carrillo-de Sauvage, et al., 2015)]. This requires an efficient strategy that i) only impacts the reactive state and not normal astrocyte functions, ii) does not affect other cell types, and iii) inhibits all (or only the targeted) classes of reactive astrocytes. With new and maybe more selective molecular markers, it will be important to extensively validate the efficiency and universality of such methods and determine how these "de-activated" astrocytes are different from non-reactive astrocytes.

Overall, the range of functional changes displayed by reactive astrocytes and their effects on disease are extremely large. They are governed by the pre-existing astrocyte molecular and functional profile, in addition to the pathological trigger itself (**Fig. 3**, **Section 5**). Indeed, even reactive astrocytes activated by the same pathway can have different effects on specific disease outcomes. STAT3 signaling in reactive astrocytes has detrimental effects in mouse models of AD (Ceyzériat et al., 2018; Reichenbach et al., 2019), or hypoxia (Hristova et al., 2016), no significant effect in the context of acute mitochondria toxicity (O'Callaghan et al., 2014) but has beneficial effects in neonatal white matter injury (Nobuta et al., 2012), after SCI, by promoting glial scar formation (Anderson et al., 2016; Herrmann et al., 2008; Okada et al., 2006), and in HD models, by reducing mutant huntingtin aggregation (L. Ben Haim, Ceyzeriat, et al., 2015). Intriguingly, a recent study by Tyzack *et al.* suggested that the upstream activator (IL6 or EphB1-ephrine-B1 signaling) controls the final molecular profile induced by STAT3 in reactive astrocytes [(G. E. Tyzack et al., 2017), **Table 2**].

It is now important to define what reactive astrocytes do in each situation and the underlying signaling cascades, before targeting these cells for therapy. There are new opportunities and yet many challenges to use reactive astrocytes for therapeutic purposes.

9. Do reactive astrocytes eventually die?

There may be extreme forms of responses leading to the loss of key astrocyte proteins and functions or even their death. Astrocytes are considered more resilient than neurons, but they may degenerate as well. As seen with TUNEL or caspase stainings, astrocytes may undergo apoptotic cell death in ischemia (Giffard & Swanson, 2005), in ALS mice (Rossi et al., 2008), or in the brain of AD patients (Perez-Nievas & Serrano-Pozo, 2018). Alternatively, mouse astrocytes may die by necrosis at the infarct core after stroke (Lukaszevicz et al., 2002).

Major alterations of astrocyte morphology, including severe process atrophy or fragmentation, swollen or vacuolated cell bodies are described in brain samples from patients with AD, stroke or infection for example, and it is called clasmatodendrosis [see references in (Perez-Nievas & Serrano-Pozo, 2018; Tachibana et al., 2019)]. It is also reproduced in animal models of these diseases (Olabarria, Noristani, Verkhratsky, & Rodriguez, 2010; Sullivan, Bjorkman, Miller, Colditz, & Pow, 2010). In addition, astrocytes may be directly or primarily targeted by the disease process, like in Alexander disease caused by *Gfap* mutation (Messing, 2018) or *neuromyelitis optica* caused by autoantibodies against the astrocyte protein aquaporin 4 [see (Verkhratsky, Zorec, & Parpura, 2017) for a complete review].

Interestingly, in a mouse model of tauopathy (Bussian et al., 2018) and in AD patients (Bhat et al., 2012), astrocytes express markers of cellular senescence ($p16^{INK4a}$ and β-galactosidase). Genetic ablation of senescent cells prevents tau pathology and cognitive decline (Bussian et al., 2018), suggesting that they have deleterious effects. It is not possible however, to know whether senescent astrocytes are responsible alone or if senescent microglia also play a role. Interestingly, interleukins IL6 and IL1β are classic senescence markers (Salminen et al., 2011) and senescent astrocytes in AD brains express high levels of GFAP (Bhat et al., 2012), suggesting that senescence could be yet another specific reactive state.

Overall, such atypical astrocyte responses characterized by major loss of key homeostatic astrocyte proteins, senescence and/or altered morphology will need to be further characterized by genome wide and refined temporal analysis to really qualify as a form of astrocyte reaction. Do astrocytes first become reactive and then adopt this dysfunctional, senescent phenotype and die, or these are distinct non-overlapping processes?

10. Where do reactive astrocytes come from or what do they become?

Reactive astrocytes are in a very distinct state from astrocytes in physiological conditions. Thus, one may wonder whether reactive astrocytes 1) can further transform into other cell types and 2) come only from the conversion of a non-reactive astrocyte. Both questions have important therapeutic implications, especially if reactive astrocytes are able to generate new neurons.

As mentioned earlier, only a subset of reactive astrocytes proliferate (*e.g.* juxta-vascular or scar-forming astrocytes), they only undergo one round of division and invariably generate two sister astrocyte cells (see **Section 4**). Strikingly, the *in vitro* situation is quite different. In a culture medium promoting cell proliferation, acutely isolated reactive astrocytes are able to generate different cell types, including neurons (Gotz, Sirko, Beckers, & Irmler, 2015). It shows that some permissive signals are lacking in the brain environment [like SHH signaling, (Sirko et al., 2013)] or conversely, that restrictive factors prevent astrocytes from returning to a multipotent state and differentiating into neurons [like Notch signaling, (Magnusson et al., 2014)]. Significant efforts are made to identify and target such factors, to promote the neurogenic potential of reactive astrocytes and their reconstruction of damaged neuronal circuits [for a complete review, see (Lei, Li, Ge, & Chen, 2019)].

Regarding the origin of reactive astrocytes, scar-forming reactive astrocytes were proposed to come from other proliferating cell types, like progenitors cells (Benner et al., 2013; Faiz et al., 2015) or ependymal cells (Sabelstrom et al., 2013). Fate mapping by Cre-reporters or viral targeting suggests that this phenomenon is marginal, with the majority of reactive astrocytes originating from previously labelled astrocytes (Buffo et al., 2008; Ren et al., 2017), but it may be insult- and region-dependent (see Gotz et al., 2015, for a complete discussion).

These two questions are intimately linked to cell identity. At present, there is no single univocal marker for brain cell types [as discussed for reactive astrocytes in **Section 6,** see (Gotz et al., 2015), for a complete discussion]. For example, GFAP is also expressed by progenitor cells. A type of thalamic glial cell expresses both Connexin43 and Olig2, which are typical astrocyte and OPC markers respectively (Griemsmann et al., 2015). Such blurred cell identities are even exacerbated in disease. Reactive astrocytes express nestin and other makers of neural stem cells (Gotz et al., 2015). Likewise, transcriptomic studies reveal that reactive astrocytes overexpress many genes characteristic of microglia (*e.g. Cst7, Trem2, Cts)* (Ceyzériat et al., 2018; Orre, Kamphuis, Osborn, Jansen, et al., 2014). Indeed, cells co-expressing astrocyte and microglia markers are observed in the spinal cord of an ALS rat model (Trias et al., 2013), the deafferented mouse hippocampus or the brain of AD, Lewy body dementia or stroke patients (Wilhelmsson et al., 2017). Moreover, reactive astrocytes may lose some of their defining functional features like input resistance, membrane currents and gap-junction coupling, as described in human brains and in a mouse model of sclerotic mesial temporal lobe epilepsy (Bedner et al., 2015). They may also express lower levels of key astrocyte proteins, as discussed earlier (**Section 4, 9**), and as shown after mild repetitive TBI with loss of GLT1, GS, KIR4.1 and even GFAP (Shandra et al., 2019). This will complicate their identification as astrocytes.

When astrocytes become reactive, they undergo profound transformations, losing their original features and "disguising" as other cells. Refined fate mapping methods with specific drivers and tight time control will be valuable to trace the origin and future of single astrocytes in disease.

11. How do reactive astrocytes interact with other innate immune cells?

The dialogue between reactive astrocytes and other cellular partners involved in inflammation and immunity (*e.g.* microglia, macrophages, lymphocytes) is quite complex. It is generally admitted that microglial cells react first to many injury signals, in particular danger- and pathogen-associated molecular patterns, and then activate

astrocytes through cytokine release (Sofroniew, 2014b). There are several reports of microglial cells driving astrocyte towards a specific reactive state. LPS-activated microglia produce IL1α, C1q and TNF and induce A1 reactive astrocytes (Liddelow et al., 2017). But microglia made reactive by methotrexate chemotherapy induce astrocytes to express A2 genes, not A1 (Gibson et al., 2019). Alternatively, when they release vascular endothelial growth factor B (VEGF-B) during EAE, microglial cells induce a possibly different deleterious type of reactive astrocyte, which needs to be fully characterized (Rothhammer et al., 2018). In TBI, microglia drives a beneficial form of astrocyte reaction through down-regulation of P2Y1 receptors (Shinozaki et al., 2017).

Overall, these studies suggest that microglia dictate the profile of reactive astrocytes, but it may go the other way around. Indeed, reactive astrocytes overexpress many cytokines, chemokines and signaling molecules that could activate neighboring microglial cells (Ceyzériat et al., 2018; Orre, Kamphuis, Osborn, Jansen, et al., 2014; Zamanian et al., 2012). Astrocyte-specific NF-κB activation induces significant astrocyte reaction that secondarily causes microglia activation (Oeckl et al., 2012; Ouali Alami et al., 2018). Accordingly, astrocytes may become strongly reactive while microglial is barely affected after viral infection (Ortinski et al., 2010), or CNTF exposure (Lavisse et al., 2012), showing that astrocytes have the ability to become reactive on their own.

Reactive astrocytes may also interact with peripheral immune cells. A recent study showed that regulatory T cells reduce astrocyte reaction (including A1 markers) after stroke, through amphiregulin, IL6 and STAT3 signaling (Ito et al., 2019). Alternatively, phospho-STAT3$^+$ reactive astrocytes found around brain metastatic cells reduce CD8$^+$ lymphocyte recruitment and increase the number of CD74$^+$ macrophage/microglia, which are more permissive to metastatic proliferation [(Priego et al., 2018), **Fig. 2R**]. Proliferating, juxta-vascular reactive astrocytes were also shown to inhibit monocyte infiltration following SWI (Frik et al., 2018). Using reporters to track expression of two genes typical of different phagocyte states, Locatelli *et al.*, also showed that astrocytes release factors that convert polarized phagocytes expressing inducible nitric oxide synthase into arginase-expressing cells *in vitro* (Locatelli et al., 2018).

Overall, reactive astrocytes engage in complex bi-directional communications with other immune cells. It remains to be determined where and when these interactions take place, as peripheral immune cells entry into the brain is tightly regulated. Microglial cells and peripheral immune cells themselves show heterogeneous phenotypes, adding yet another layer of complexity to understand the role of reactive astrocytes in disease (Song & Colonna, 2018).

12. Are aging astrocytes a type of reactive astrocytes?

The human aging brain displays higher GFAP immunoreactivity and mRNA levels [see references in (Munger et al., 2019)], and it was assumed that aging causes progressive astrocyte reaction. But this idea is not consensual (see **Table 1a**). It is possible for example that the analyzed subjects were exposed to some pathological stimuli (mild trauma, micro-infarcts, inflammation or infection) that caused GFAP increase, not aging itself. Again, transcriptomics studies in mice provide a fuller view on what happens to astrocytes with aging. Results showed that aging astrocytes express higher levels of genes linked to immunity and inflammation (Boisvert et al., 2018; Orre, Kamphuis, Osborn, Melief, et al., 2014). Another study showed that 2-year-old astrocytes express several pan and A1 genes at higher levels than young astrocytes (Clarke et al., 2018). Aging astrocytes also display reduced expression of some genes linked to cholesterol or ROS detoxification (Boisvert et al., 2018; Clarke et al., 2018). But overall, the molecular changes occurring in aging mouse astrocytes are rather limited. Interestingly, there are regional differences, with larger variations in hippocampal and striatal astrocytes than in their cortical counterparts, even if *Gfap* is induced in all brain regions (Boisvert et al., 2018; Clarke et al., 2018). A genome-wide study in Humans also evidenced significant, region-dependent changes in astrocyte-specific genes with aging (Soreq et al., 2017).

When analyzed in a healthy aging brain, astrocytes display mild reactive changes. However, aging astrocytes have a high probability to have encountered previous pathological conditions in their lifetime, and they may thus be in a primed state that will change their subsequent response (Cunningham et al., 2018). Astrocyte reaction

and possible priming in the aging brain need to be thoroughly analyzed, as aging is a major risk factor for several brain diseases.

13. Which new approaches and tools will move the field forward?

There are now many techniques and models to study astrocytes, some mentioned in the previous paragraphs [*e.g.* new transgenic mice, purification methods, human induced pluripotent stem cells (iPSC)-derived astrocytes (Almad & Maragakis, 2018; Guttenplan & Liddelow, 2019)]. In addition, refined cellular and molecular methods developed in other fields could prove very useful to study reactive astrocytes like spatial transcriptomics (Rodriques et al., 2019; Wang et al., 2018), scRNAseq (Svensson et al., 2018), Cas9 genome editing and screening (Shalem, Sanjana, & Zhang, 2015) and multiplexed immunostainings (Goltsev et al., 2018). Miniaturization (in terms of quantity of input sample), extension to large brain regions and multiplexing to hundreds or thousands of target genes or proteins will achieve unprecedented resolution to decipher how astrocytes react in a given disease context and define better molecular markers (see **Section 6**). But in the end, it is the function that matters. How will these molecularly defined reactive astrocytes perform their normal functions? This is easier tested *in vitro* where several functions can be quickly screened, [*e.g.* phagocytosis, glutamate uptake, catabolism of specific metabolites, production of cytokines/trophic factors/complement factors, toxicity towards other cell types, effect on synapses, (Diaz-Amarilla et al., 2011; Escartin et al., 2007; Liddelow et al., 2017; G. E. Tyzack et al., 2014)]. It may be trickier to explore astrocyte functions *in vivo* or at least in *ex vivo* settings like acute brain slices or acutely dissociated astrocytes. It is important to combine functional assessment with some morphological or molecular markers to ascertain that probed astrocytes are indeed reactive and ideally provide some insight into their molecular features. Therefore, methods with cellular resolution like electrophysiology combined with fluorescent detection of markers, multidimensional cytometry and two-photon imaging with functional sensors are methods of choice to establish how specific types of reactive astrocytes function. Several elegant studies recently succeeded in providing new insight into the complexity of astrocyte functions in the normal brain (Bindocci et al., 2017; Chung et al., 2013; Martin, Bajo-Graneras, Moratalla, Perea, & Araque, 2015; Nimmerjahn & Bergles, 2015) and in disease (Bardehle et al., 2013; Delekate et al., 2014; John Lin et al., 2017; Yu et al., 2018). Future studies will need to implement such refined methods in relevant disease models, to study reactive astrocyte responses at the single-cell level.

Importantly, it is now clear that human astrocytes are more complex than their rodent counterparts and express specific genes (Oberheim et al., 2009; Y. Zhang et al., 2016). *In vitro,* human and mouse astrocytes are reactive to different stimuli and overexpress different genes [(Tarassishin, Suh, & Lee, 2014), **Table 2**]. It is thus important to eventually go back to human samples to confirm findings obtained in animal models (Arranz & De Strooper, 2019). Patient brains are a valuable resource but with associated bias due to *post mortem* delay, comorbidities and other confounding factors. Derivation of human astrocytes from iPSCs taken from patients is opening new research opportunities to probe disease-specific changes (G. Tyzack, Lakatos, & Patani, 2016). However, the study of reactive astrocytes may be particularly difficult, as iPSC-derived astrocytes (and cultured astrocytes in general) tend to be already reactive, especially when exposed to serum. It is thus required to optimize differentiation and culture protocols to obtain a mature phenotype, reactive to subsequent stimulation (Perriot et al., 2018). It will also be important to define which subtype of reactive astrocytes is modeled and to confirm that this subtype truly exists *in vivo*.

Research on astrocytes is at an exciting stage, with potent approaches, new models and refined concepts to study their role in the normal and diseased brain.

Concluding remarks

It is now becoming obvious that broad and quite old expressions like *Astrocyte reactivity* or *Astrogliosis*, hide in fact a striking complexity with subtle regulations. Our survey highlights that several aspects of astrocyte reaction are still unclear or even controversial, starting with their very name. But there is a strong interest in better understanding this specific transformation of astrocytes in response to a pathological stimulus. Reactive

723 astrocytes may have significant impact on disease progression, diagnosis or even treatment. Therefore, we
724 should now aim to disentangle this widespread and complex brain response to disease and define which aspects
725 are amenable to therapeutic improvement.

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Figure legends

Figure 1. Original drawings of reactive astrocytes in Humans

I-II.(P. Chaslin, 1891). Chaslin's drawings of sclerotic cortical brain sections from an adult epileptic patient, impregnated by the picro-carmin method. **I.** This drawing represents a non-lesioned region. A blood vessel (d) connected by a star-shaped astrocyte (c) is observed, as well as few small nuclei. **II.** The drawing of a lesioned tissue shows an accumulation of large bundles of fibers and many nuclei, characterized by Chaslin as "gliotic tissue". **III-VII**.(Ramón y Cajal). Ramon y Cajal's drawings of cortical (**III-VI**) and cerebellar (**VII**) brain sections from an adult patient suffering from general progressive paralysis, after silver impregnation. **III.** A reactive astrocyte with thick branches polarizing towards a blood vessel (a). Thin processes emerge from the other side of the cell body (b). Microglia cells (e, d) and one neuron (c) are also visible. **IV.** Large bundles of fibers named "Weigert fibers" (A), emanating from glial cells, which are barely visible. Note the similitude with Chaslin's drawing (**II**). C: microglia. **V**. Reactive astrocytes with twisted processes (a, b, c, d) surrounding an infiltration nodule (A). Degenerating neurons with protein aggregates in their cell bodies are visible (G). D: blood vessels; f: astrocyte endfeet. **VI.** Reactive astrocytes with numerous polarized processes have lost their individual, non-overlapping domains. Astrocyte cell bodies are sometimes in direct contact, suggesting "proliferative astrocyte centers" (A, C, E), as proposed by Ramon y Cajal. D: blood vessels. **VII.** Hypertrophic cerebellar astrocytes (B, C, D). Two of them (C, D) have multinuclear cell bodies, suggesting ongoing cell division. b: blood vessel.

Figure 2. Astrocytes become reactive in a variety of pathological conditions and species.

Different methods are used to evidence astrocyte reaction to different diseases, in different species and brain regions, showing the universality of this response but also some context-specific changes. **A.** Frontal cortex mouse brain section following SWI. A gradient of reactive astrocytes ($GFAP^+$, green) is visible around the injury site. Scale bar: 500 µm (Frik et al., 2018). **B.** Reactive astrocytes in the cortex of a mouse EAE model. Reactive astrocytes are labeled with StarTrack multicolor lineage tracing system to identify developmental clones by their pattern of fluorescent protein expression. Scale bar: 80 µm (Bribian et al., 2018). **C.** Immunofluorescence for GFAP and S100β (green), Ki67 (white) and CD31 (blood vessels, red) in the mouse cortex, 5 d after MCAO. Several reactive astrocytes are proliferating ($Ki67^+$) in the ischemic penumbra (yellow arrowheads) (Frik et al., 2018). **D.** 3D rendering of the morphology of a normal (blue) and a hypertrophic CNTF-induced reactive astrocyte (brown) in the mouse striatum. Scale bar: 50 µm (Ligneul et al., 2019). **E.** A non-reactive (up) and reactive (down) astrocyte in the mouse cortex, 4 d after cortical lesion. Dye-filling reveals the astrocyte ramified morphology and process enlargement in reactive astrocytes. Scale bar: 25 µm (Wilhelmsson et al., 2006). **F.** Immunofluorescence for GFAP (cyan) and BrdU (red) in the mouse spinal cord after SCI. The border of the lesion core (upper part) presents a high proportion of $BrdU^+/GFAP^+$ proliferative reactive astrocytes forming the glial scar. Scale bar: 25 µm (Wanner et al., 2013). **G.** Immunoelectron micrograph of a phagocytic reactive astrocyte in the mouse striatum 3 d after MCAO. GFAP staining is outlined in blue. Phagocytic inclusions (arrowheads) are visible. Scale bar: 2 µm (Morizawa et al., 2017). **H.** Two-photon microscopy of GLAST-eGFP mouse cortex, 28 d after SWI. A GFP^+ astrocyte undergoes cell division (green arrowheads), while some of its processes (yellow arrows) polarize towards the lesion (yellow star). Scale bar: 20 µm (Bardehle et al., 2013). **I.** A dextran-filled astrocyte in a rat transected optic nerve. This reactive astrocyte displays increased caliber of individual fibers and loss of global complexity. Scale bar: 30 µm (Butt & Colquhoun, 1996). **J.** Image of 2 adjacent GFP^+ astrocytes (white) labeled with DiI (green) and DiD (red) in the mouse cortex, 1 w after iron injection to induce epilepsy. Their processes overlap more (box), showing partial loss of domain organization. Scale bar: 20 µm (Oberheim et al., 2008). **K.** Reactive astrocytes around an amyloid plaque in the hippocampus of 3xTg-AD mice (GFAP in green and Aβ in red). Scale bar: 20 µm (Olabarria et al., 2010). **L**. Reactive astrocytes (GFAP, green), around a vessel overexpress the chemokine CCL2 (red) in a mouse model of prion disease (ME7), 2 h after TNFα injection in the hippocampus (Hennessy et al., 2015). **M-M'**. Electron micrographs of a CNTF-induced reactive (M') and a control (M) rat astrocyte. The reactive astrocyte displays an enlarged process filled with bundles of filaments (black star). Scale bar: 1 µm (Escartin et al., 2006). **N.** A human perivascular reactive astrocyte (GFAP, brown) close to a leukoencephalopathy lesion contains phagocytosed LFB^+ myelin debris (blue, arrow). Scale bar: 25 µm (Ponath et al., 2017). **O.** Triple immunofluorescence for STAT3 (green), GFAP (red), and Huntingtin aggregates (magenta) in the putamen of a macaque lentiviral model of HD. Reactive astrocytes display STAT3 nuclear labeling. Scale

bar: 40 µm (L. Ben Haim, Ceyzeriat, et al., 2015). **P.** Reactive astrocytes around an amyloid plaque in the hippocampus of an AD patient, visualized by GFAP (black) and Aβ (brown) immunohistochemistry. Note similarity with image in K. Scale bar: 50 µm (Pike, Cummings, & Cotman, 1995). **Q.** Electron micrograph showing an enlarged perivascular reactive astrocyte endfoot enclosing a cortical vessel (V) in the brain of an AD patient. x 7,000 (Wegiel & Wisniewski, 1994a). **R.** Human cortical reactive astrocytes labeled for GFAP (green) and phospho-STAT3 (red) around brain metastases from lung cancer (dotted lines). Scale bar: 35 µm (Priego et al., 2018). **S-S'.** GFAP immunohistochemistry on *subtantia nigra pars compacta* sections from a control and a parkinsonian macaque treated with MPTP (C. Barcia et al., 2011). **T.** Reactive astrocytes in acute lesions of a MS patient. Triple immunofluorescent staining of reactive astrocytes with GFAP (green), Complement 3 (C3, red) and activated microglia/macrophages with CD68 (white). Scale bar: 100 µm (Liddelow et al., 2017). **U.** Immunofluorescence staining for S100β (green) and *in situ* hybridization for C3 (red) in an ALS patient. Scale bar: 10 µm (Liddelow et al., 2017). **V.** 3D rendering of a reactive astrocyte stained with vimentin around human glioblastoma. Scale bar: 35 µm (C. Barcia, Sr. et al., 2013).

Figure 3. Heterogeneity of reactive astrocytes: causes and manifestations

Normal astrocytes are already heterogeneous due to different sources of diversity [listed in blue, see (L. Ben Haim & Rowitch, 2017), for references]. Upon injury or disease, astrocytes display even more heterogeneity (listed in brown), depending on the exact molecular trigger (*e.g.* open versus closed injury, extracellular cytokines versus intracellular aggregated proteins, aberrant neuronal activity versus cell death), their distance from it (topography), the exposure time to this stimulus (disease stage) and the signaling cascades activated. The combination of pre-existing and induced heterogeneity gives rise to potentially very diverse populations of reactive astrocytes with unique molecular and functional features. However, there are some core changes that are common to most forms of reactive astrocytes (*e.g.* GFAP induction, morphological changes, represented in red).

Table legends

Table 1: Reactive astrocytes: definitions, consensus and controversies.

In December 2018 and January 2019, we surveyed 61 researchers actively working in the field of (reactive) astrocytes with different approaches (molecular, morphological, and functional), on different diseases and models or in physiology. We asked them the following two questions:

*a- What is your definition of astrocyte reactivity, in a few sentences? (**Responses listed in Table 1a**)*

*b- Do you consider "astrogliosis", "astrocyte activation" and "astrocyte reactivity" as synonymous terms? (**Responses listed in Table 1b**)*

We collected 38 replies (see **Supplemental Text** for full replies) from junior and more senior researchers, from both genders and 12 countries (see list in **Supplemental Table 1**). It is not a perfect or unbiased sampling of the community, but it represents the first attempt to survey different researchers working and publishing on astrocytes. It shows that some points are consensual while other are more controversial.

Replies were analyzed and grouped under different features or concepts (1st column), addressing different questions listed in gray boxes above. Respondents, identified by their initials**,** either agreed to these definitions (2nd column), agreed and provided further explanations (3rd column) or disagreed (4th column). Pie charts represent the percentages of responses (green = agree; red = disagree; gray = not mentioned) for features mentioned by more than 18 respondents or for controversial features.

The definitions given in response to question **a** are linked to the nomenclature discussed in response to question **b**, since several researchers provide specific definitions for each term. For the sake of simplicity, we considered

that responses to question **a** may apply to any of the three terms (astrogliosis, astrocyte activation, and astrocyte reactivity). Responses to question **b** help better pin down further differences linked to terminology.

In Table 1a: Glu, glutamate; ND, neurodegenerative diseases; NSC, neural stem cells.
In Table 1b: * Active astrocytes = contain proBDNF with no GABA, hypertrophy. Reactive astrocytes = elevated MAO-B, high GABA, low proBDNF. Astrogliosis = severe reactive astrocyte, with proliferation.

Table 2. Astrocyte reaction is a heterogeneous response

This table presents a selection of relevant studies, performed mostly *in vivo,* to illustrate heterogeneity of astrocyte reactions. Different levels of observed heterogeneity can be listed as originally proposed by (Anderson et al., 2014). In addition, differences in responses are observed depending on disease stage or age (temporal), species, and gender. Interestingly, several levels of heterogeneity may co-exist (see Additional levels of heterogeneity). Most of the time, the reactive state of astrocytes was established by GFAP overexpression and morphological changes. Morphological changes were estimated qualitatively (visual), unless mentioned in the table. Some studies identified additional markers of (subtypes) of reactive astrocytes.
Abbreviation list (by lexical field): ***CNS Regions****: Cb, Cerebellum; Cx, Cortex; Hip, Hippocampus; SC, Spinal cord; Str, Striatum; Th, Thalamus; WM, White matter.* ***Diseases****: AD, Alzheimer disease; ALS, Amyotrophic lateral sclerosis; MS, multiple sclerosis; NWMI, neonatal white matter injury; Neuroinfla., neuroinflammation; PD, Parkinson disease; SCI, Spinal cord injury; TBI, traumatic brain injury.* ***Experimental models****: EAE, experimental autoimmune encephalomyelitis; KA, kainate; LPS, lipopolysaccharide; MCAO, Middle cerebral artery occlusion; SWI, stab wound injury.* ***Species****: H, Human; Mc, Macaque; Mm, Mammalian; Ms, Mouse; R, Rat.* ***Other fields****: Astro: astrocytes; CSPG: chondroitin sulfate proteoglycans; IHC/IF, Immuno-histochemistry or -fluorescence; IR, Immunoreactivity; WB, Western Blotting; =, unchanged; ↗, increase.*

Supplemental Material

Supplemental Table 1

List of all surveyed PIs with their affiliation and initials used in **Table 1a** and **1b**.

Supplemental Text 1

Full answers of all surveyed PIs (list by alphabetical order), to the two questions:

a. *What is your definition of astrocyte reactivity, in a few sentences?*
b. *Do you consider "astrogliosis", "astrocyte activation" and "astrocyte reactivity" as synonymous terms?*

Author contribution

CE wrote the review, performed the survey and generated Table 1a, Table 1b and Fig 3. OG generated Table 2. MACS produced Fig 1 and 2. All authors edited and finalized the review.

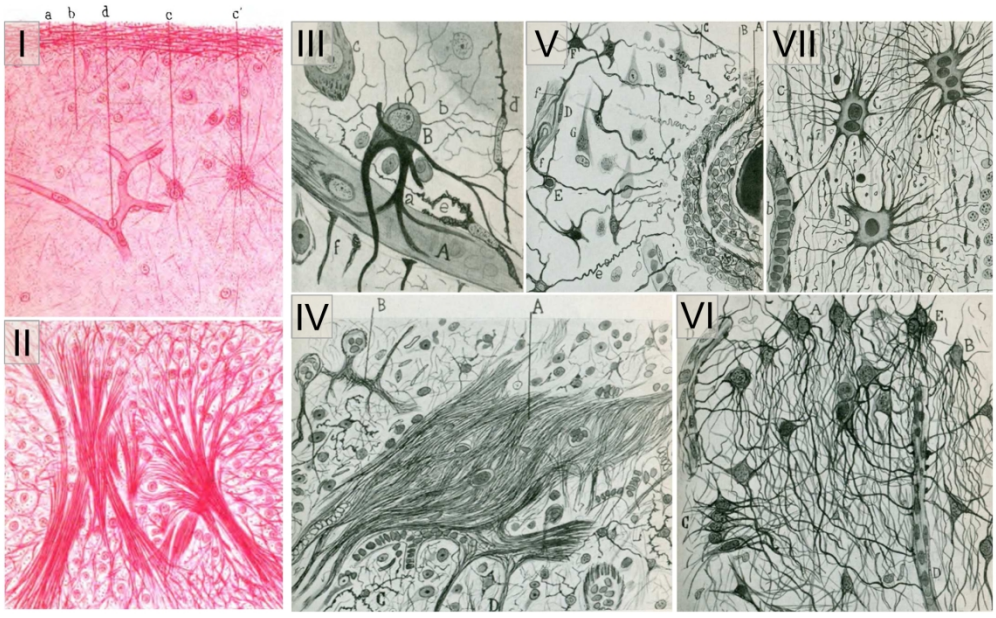

Figure 1

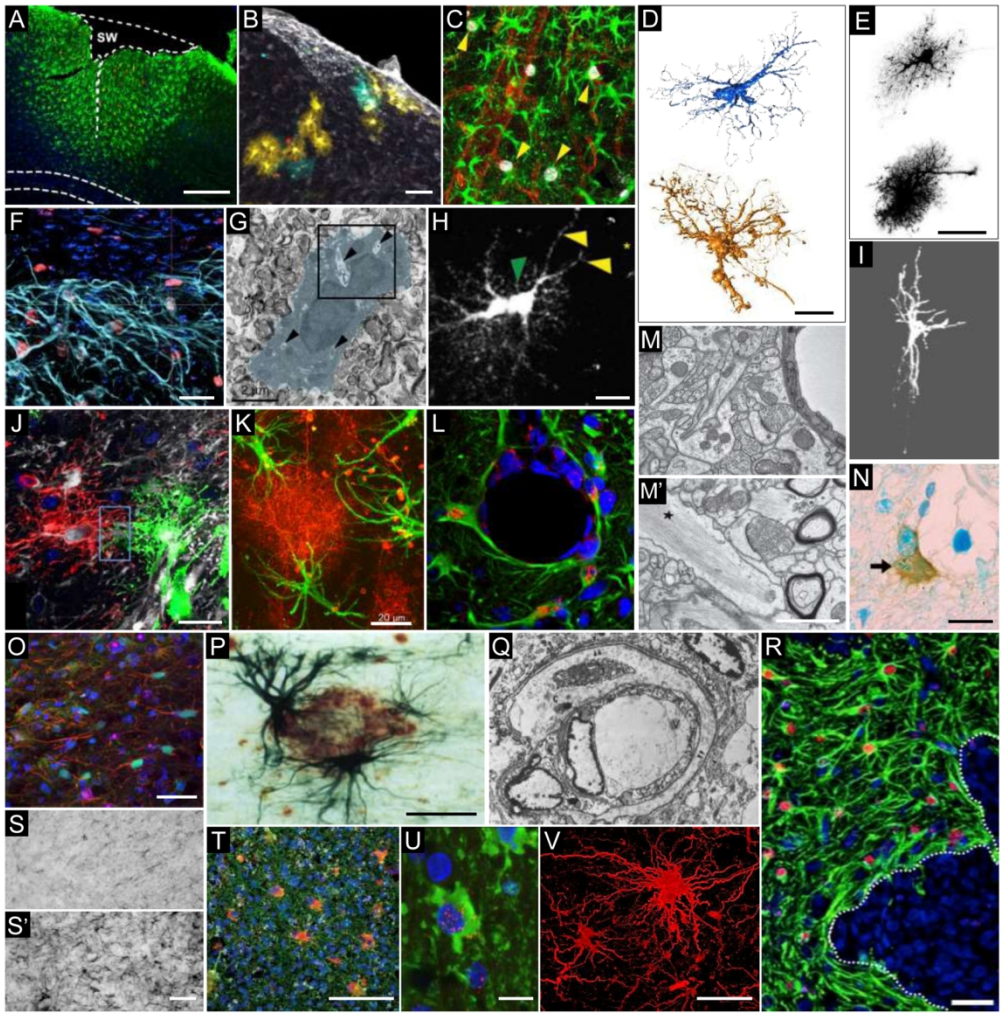

Figure 2

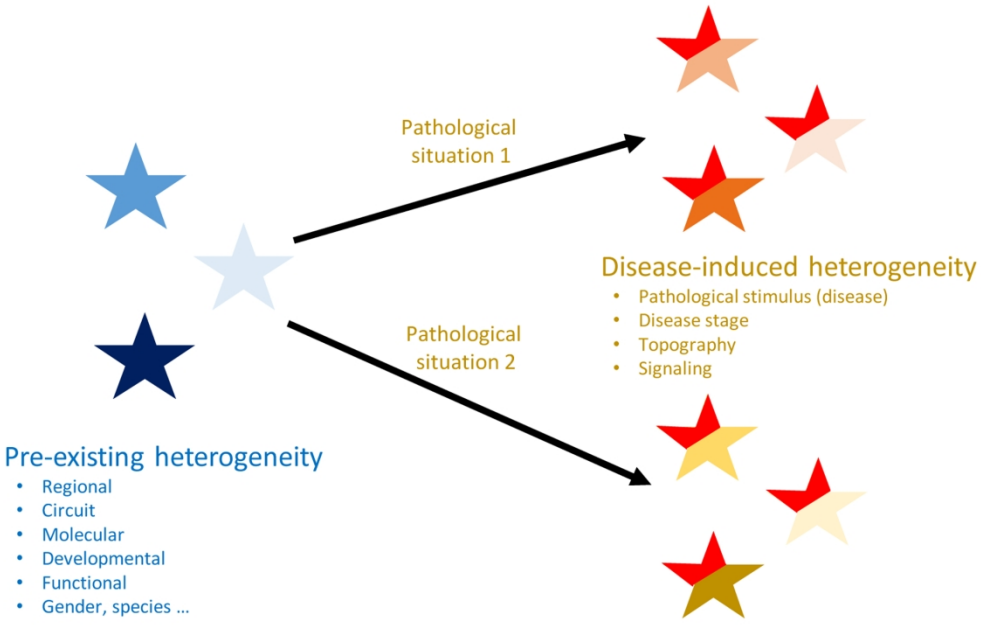

Figure 3

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TABLE 1a: Definitions of astrocyte reactivity

Feature/concept	Agree	Agree & Refine	Disagree	
What is it?				
A phenotypic change, alteration	**AA AM ASP AP AVo CC CS EG FP GC HK HS LP MF MS NA PH SHRO SL SR VG**	**GaP:** short term changes also (Ca^{2+}, Glu/GABA/K^+ buffering, coupling..) **KM:** dynamic state **IW:** changes of viable, not wounded/dying astrocytes		
A response	**AL AM ASP EG FP GC HS IW JOC MF MG MS NA SL SHRO SO SS VG**			
A reprogramming, transformation, conversion	**AVe KM**	**ASP:** genes & functions down-regulated as well **AVo:** go back to more immature/plastic state **DB:** may come from latent progenitors **SK:** become a different type of cells		
An ability, propensity	**SS ASP SO**	**CC:** they are now more reactive to a future stimulation **LP:** capacity to become		
What does it change in astrocytes?				
Molecular (mRNA & proteins), morphological, functional changes	**AA AM AVo ASP CS EG FP GC HK HS JOC LB MS NA SK SR SS VG**	**AP:** reactive astrocytes integrate & regulate information differently **CN:** initial response biochemical, then transcriptional **SHRO:** biochemical as well **GaP IW KM:** Activation of specific signaling pathways		
Both gain & loss of functions	**ASP CN MS**	**GC:** importance of Ca^{2+} signalling **HS:** global loss of proteins involved in ion/neurotransmitter homeostasis & change in extracellular matrix production **SHRO:** gain/loss of function can occur without reactive phenotype		
Proliferation not prevalent	**ASP KM LP**	**EG:** may refer to GFAP$^+$ NSC converting into astrocytes **MS:** newborn astrocytes form scars		
What induces it?				
Non-physiological conditions (injury, disease, stress, environment alterations)	**ASP CN CS EG FP GC JOC HK HS IW MS NA SL SO SR VG**	**AL GeP MF SHRO :** not exclusively pathological stimulus **AM:** exclude any physiological changes		
External/environmental stimulus or signal	**AL ASP GaP MF SHRO**	**JOC:** in response to glia injury as well = internal signal		
Aging also	**ASP SR**		**CS:** aging is not necessarily pathological	
Is it a homogeneous response?				
Heterogeneous:	**AL AM ASP AVe AVo CC CJL CS CN DB DR EG GaP GC HK JOC KM LB MF MG MS NA PH RS SL SS**			
- continuum	**GaP MF PH**	**AVe MS SS:** tuned to the nature & strength of the insult **AVe CS DB:** more than A1/A2 **CS:** worsens with disease progression **DB:** worsens with age	**AVo AM CJL MG SL:** discrete states/phenotypes rather than continuum	
- dictated by type of insult/disease ...	**AM ASP EG GC HK MF MG NA SL**			
... and by previous heterogeneity, brain regions, age	**AVe AVo CS CN KM SS**	**DB MS:** also by signaling		
GFAP & morphological changes are core hallmarks/defining features	**AA DR EG GaP JOC KM MF NA PH RS**	**LP:** also associated with increased cytokines	**ASP:** morphological changes not systematic **AVo:** hypertrophy may be an artifact of increased GFAP staining **AVe CJL RS:** misleading **CJL:** GABA-based classification instead **GC:** charaterize only astrogliosis	
What are the consequences?				
Can have good or bad effects	**DR GC LP MS SK**	**EG:** adaptive response in acute injury to recover homeostasis by sealing off injured tissue. In chronic injury, maladaptive with dysfunctional astrocytes **GaP:** not only secondary, also key contributor	**AVe:** always good, evolutionary driven. Even neurotoxic astrocytes may have defensive roles **AVe HS IW SO:** objective = restore homeostasis **IW:** form a scar to limit lesion expension **CS:** reactive astrocytes are pathologically altered **CJL:** always bad in ND through GABA/H_2O_2 release	
Reversible & transient	**CN GC MS SO**	**GeP SS:** astrogliosis is not		

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TABLE 1b: Nomenclature *(astrogliosis, astrocyte activation, astrocyte reactivity)*

	Agree	Agree & Refine	Disagree	
These 3 terms are ill-defined...	**AM CC CS DB DR FP MG PH SO**	**EG RS**: meaningless terms	**MS**: still valid	
...need **new terminology** or at least **precise definition**	**ASP AVe AVo CIL DB GaP LB MG**	**EG**: based on molecular & functional profiles	**SL**: better not add new names	
Use a simple, broad, inclusive, collective definition	**MF MG MS SR**	**AL CC DB**: but then need to precise categories/functions/specification **GaP**: umbrella definition		
These 3 terms have different meanings	**ASP AVo CC CN GC GaP LB MG PH SS**	**CIL**: GABA based classification for hippocampal/cortical astrocytes.*		
... refer to **different grades/stages**		**CN**: reactivity = biochemical response, maintained as activation (transcriptional) **GaP MG SS**: increase severity: activation < reactivity <. astrogliosis **GeP**: activation = reponse to normal neuronal activity, reactivity = reponse to abnormal neuronal activity **LB**: Astrocyte activation = functional/phenotypic changes. Astrocyte reactivity = morphological changes **LP VG**: activation = transition to reactivity	**All 3 equivalent** **AVe DB HK IW JOC RS SL** . **AVe**: also "Astrocytosis"	
Astrocyte activation refers to **physiology** and **should not be used** for pathology	**AA AM CS EG FP GC HS NA SR SS**	Not appropriate, because: **ASP**: suggests independent of a trigger. May involve loss of function **KM**: better suited for microglia **MS SL**: suggests inactive otherwise	**Activation = Reactivity** **AL AP DR MF SK SO** **AL**: both involve response to stimulus (either physiological or pathological)	
Astrogliosis is different :	**FP**	**ASP**: astrogliosis should be abandoned [-osis = pathological state, proliferation/increased number or invasion/infiltration/spreading]	**Astrogliosis = Reactivity** **AA CS EG HS MS NA SR**	
- involves **proliferation or increased numbers**	**AL ASP CC CN LP LB MF SK VG**	**CC**: property of a population		
- involves **scar**	**GaP KM SO**	**DR**: clinical term = injury		
- involves **inflammation** (= immune cell recruitement)	**AL MG**	**AP**: increase in 3D domain		
- involves **hypertrophy**	**LB**	**GC**: with GFAP expression		
- **ultimate** stage	**AL AP**	**GeP SS**: irreversible		

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Levels of observed heterogeneity	Disease modeled	Experimental model	CNS region	Species	Examples of heterogeneity	Additionnal levels of heterogeneity	Hypertrophy observed? Method used	↗ GFAP observed? Method used	Others markers of reactive astro	Reference
Species	Neuroinfla.	Primary astro exposed to LPS, IL1, Poly IC		H, Ms	Only Ms astro are dependent on TLR4 or CD14 Ms & H reactive astro produce different cytokines	Signaling	n.m.	n.m. (↗ cytokine)		Tarassishin 2014
	PD	MPTP (Mc), 6-OHDA (R)	Str	Mc, R	Mc: ↘ Cx30 IR; R: ↗ Cx30 IR		visual	IHC/IF		Charron 2014
Gender	Stroke	MCAO	Cx	Ms	↗ GFAP IR F>M		visual	IHC/IF		Cordeau 2007
	Trauma	SWI	Cx	Ms	Nbr $Ca2^+$ astro around lesion M>F		visual	IHC/IF	Vimentin	Acaz-Fonseca 2015
Disease	AD, epilepsy	Tg2576, KA	Cx	Ms	Loss of astro domain organization in epilepsy but not in AD	Morphological	Diolistic labeling	IHC/IF		Oberheim 2008
	Trauma, stroke, AD	SWI, MCAO, APP/PS1	Cx	Ms	Shh promotes astro proliferation & stem cell potential	Signaling	visual	IHC/IF		Sirko 2013
	Neuroinfla., stroke	LPS, MCAO	Cx	Ms	50% regulated genes are disease-specific	Molecular	visual	IHC/IF	*Serpina3n, Lcn2*	Zamanian 2012
	Trauma, AD	SWI, APP23	Cx	Ms	Different GFAP mRNA ↗ (TBI: 82-fold, denervation: 30-fold, AD: 18-fold increase)		visual	IHC/IF, qpCR		Berbesh 2004
Temporal	ALS	$SOD1^{G93A}$	SC	Ms	Presymptomatic stage: astro NF-KB activation délays disease progression. Symptomatic: accelerates disease progression		visual	IHC/IF		Ouali Alami 2018
	MS	EAE	Cx, Hip, Cb, SC	Ms	Early stage: ↗ MHC-II mRNA, ↘ cholesterol synthesis pathway Late stage: ↗ MHC-II mRNA, ↘ cholesterol synthesis pathway Gene expression changes differ between the 4 regions	Regional, Molecular	n.m.	qPCR		Itoh 2018
Regional	AD	astro cultures exposed to βAPP	Cx, Hip, Cb, SC	R	Reaction induced only in Cx & Hip		visual	IHC/IF	CSPG	Höke 1994
	Epilepsy	Pilocarpine	Hip, Cx	Ms	Different transcriptional changes between Hip & Cx astro	Disease stage Molecular	visual	IHC/IF, qPCR		Clasadonte 2016
Topographical	AD	/	Cx	H	↗ Cx43 IR around plaques		visual	IHC/IF, EM		Nagy 1996
	AD	$APP_{swe}/PS1_{dE9}$	Cx	Ms	↗ P2Y1R mediated Ca^{2+} signaling in reactive astro near plaques	Signaling	visual	IHC/IF		Delekate 2014
	Trauma	SWI	Cx	Ms	Proliferation of juxta-vascular astro only	Functional	visual	IHC/IF		Bardehle 2013
	SCI, trauma	Laminectomy L1/L2, Cryo-injury	SC, Cx	Ms	Scar border: elongation & proliferation (↗ radial glia markers) Distal: no proliferation & hypertrophy (↗ GFAP)	Morphological, Functional	Morphometry	IHC/IF		Wanner 2013 Kim 2012
	Trauma/Excitotoxicity	SWI/KA		Ms	$NFATc3^+$ astro are found only around lesions	Topographical	visual	IHC/IF	NFATc3	Serrano-Pérez 2010
Morphological	AD	3xTg AD	Hip	Ms	Astro atrophy away from plaques (↘ GFAP+ surface & volume)	Topographical	Morphometry	IHC/IF		Olabarria 2010
	Trauma	Needle-induced lesion	Cx	Ms	At injury site: mesh like morphology & small cell bodies. At the border: enlarged soma & thick processes	Topographical, Functional	visual	IHC/IF		Martin-Lopez 2013
	TBI	Lateral fluid percussion	WM, Hip, Cx, Th	R	Intertwined processes in Hip & Cx; Thick shortened processes in Th. No vimentin induction in Th	Regional Disease stage	visual	Cajal gold sublimate stain, dot-blot	S100β, Vimentin	Hill 1996
Signaling	Peripheral nerve injury	Purified astro treated with EphB1 or IL6	Cx	Ms	EphB1 (but not IL6) induces protective transcriptional profile in astro via STAT3 signaling	Molecular, Functional	visual	IHC/IF, WB	EphB1/STAT3	Tyzack 2017
	Stroke	MCAO	Cx	Ms	Only Notch1-STAT3-ETBR$^+$ reactive astro proliferate	Functional	visual	IHC/IF, WB	Jagged1	LeComte 2015
	Stroke	Photothrombosis	Cx	Ms	Only $Thbs4^+$ reactive astro proliferate in SVZ via Notch1-Nfia signaling	Functional	visual	IHC/IF	Thbs4	Benner 2013
	Trauma	SWI	Cx	Ms	Only Galectin 1 & 3$^+$ reactive astro proliferate	Functional	visual	IHC/IF	Galectins	Sirko 2015
	SCI	Contusion	SC	Ms	Type I collagen$^+$ astro induce scar formation via N-cadherin pathway pathway. Not active in hypertrophic reactive astrocytes	Topographical, Molecular, Functional	visual	IHC/IF, WB	*Cdh2, Ctnnb1…*	Hara 2017
Molecular	Neuroinfla.	Immunopanned astro LPS treated mice		Ms	"A1" neurotoxic reactive astro induced by microglia. "A2" reactive astro express different genes, function uncharacterized	Functional	visual	IHC/IF, qPCR	A1 & A2 panels + pan markers	Liddelow 2017
Functional	NWMI	Hypoxic-ischemic encephalopathy	Cx	H	"A2" reactive astro induce OPC maturation arrest via COX2-PGE2 signaling		visual	IHC/IF, qPCR	COX2	Shiow 2017
	Brain metastasis	Tumor resection	Cx	H	$pSTAT3^+$ astro reduce immune cell recruitement to metastases	Signaling	visual	IHC/IF	pSTAT3	Priego 2018
	MS, multifocal encephalopathy	/	WM	H	Some reactive astro uptake myelin & produce more cytokines		visual	IHC/IF		Ponath 2017

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Supplemental text 1

Dr. Nicola Allen

1) Definition: An alteration in astrocyte properties in response to a detrimental insult, for example an infection, a lesion or a stroke. There are core changes that define a reactive astrocyte that happen in response to all insults e.g. upregulation of GFAP, and others that are specific to the type of insult.
2) Astrogliosis and astrocyte reactivity are interchangeable; I find astrocyte activation too vague, as it could also apply in a healthy situation where an astrocyte responds to a stimulus for example with an increase in intracellular calcium.

Prof. Alfonso Araque

1) Astrocyte reactivity is a phenotypic alteration manifested as structural changes of astrocyte morphology (e.g., enlargement of cellular processes) and expression regulation of proteins (e.g., GFAP).
2) I consider astrogliosis and astrocyte reactivity as synonymous terms. Others would may consider astrocyte activation as synonymous as well. However, as physiologist, I prefer to consider astrocyte activation referred to cellular activity, i.e., astrocyte are activated by signaling molecules such as neurotransmitters, which acting on transmitter receptors activate signaling pathways that regulate cellular activity (e.g., regulating the calcium-based cellular activity that is characteristic of astrocyte physiology).

Prof. Luis Barbeito

It's true that the terms we use to describe astrocyte functional or morphological states are somewhat confusing, overlapping and conceived with an "old mind" that must be updated.
In my understanding "Reactive astrocytes" preferably refers to a morphological feature of A (hypertrophy, long processes, etc).
It also involves GFAP overexpression although this is phenotyping. The definition is linked to the concept of primary or secondary cellular or tissue "damage" or dysfunction, with the potential to induce "activation".
Then, reactive astrocytes can be subdivided into multiple subsets by applying immunophenotyping, transcriptome analysis or functional profile, which results in multiple and heterogeneous types of cells. That's the complexity we currently face.
While astrocyte reactivity is a morphological feature, astrocyte activation is mostly functional or phenotypic. Could be a link between both but refer to different attributes.
Astrogliosis comes from classical neuropathological observations and implies an increased number of astrocytes and hypertrophic.

Prof. Dwight Bergles

This sounds like a very worthwhile project, one that will help the field adopt more specific terminology and perhaps spur the development of additional tools to assess phenotypic changes in astrocytes. Although assigning cells to discrete states (e.g. M1 and M2 for microglia and A1 and A2 for astrocytes) is a useful construct and clearly encompasses shared features among cells, I expect that reactivity exists on a continuum, with cells exhibiting different phenotypic changes relative to the timing of the insult (or state of disease) and proximity to other pathology. As we learn more about the diversity among astrocytes, we may find that different regions or different "classes" of astrocytes have different capacities to alter specific pathways associated with a "reactive" status. Likely, such differences will be magnified by age. In addition, some reactive astrocytes that appear in the CNS may also be derived from latent progenitors, rather than through transformation of existing astrocytes.

Regarding the terms, I guess I would group these terms together. But, as they are so vague, they don't really carry much value by themselves without additional quantitative descriptors of what criteria were used to assign cells to this category.

Prof. Giorgio Carmignoto

1. I would say that the reactivity of astrocytes, i.e. the change in morphology and function in response to unphysiological situations, such as brain trauma, stroke or diseases, represents a striking example of the high complexity of the astrocytic role in the brain.
Reactive astrocytes lose their physiological roles and acquire new actions. Certainly, however, we have not a clear understanding of how these changes develop under pathological conditions and, most importantly, whether and to what extent these astrocytes oppose, or favor, the progression of a specific disease (see Bussian et al . Nature 2018).
An important aspect is the calcium signalling that is also affected in reactive astrocytes (in Parkinson, Alzheimer's disease etc….). Given that it regulates the release of gliotransmitters, inflammatory agents etc... its alteration affects the reciprocal communications between astrocytes and other cells, including neurons and microglia. The role of astrocytic calcium signal dysregulations in disease onset and progression remains poorly understood. This is, in my opinion, an important issue that can be adequately addressed in future studies also thanks to genetically encoded calcium indicators.
I would add that the "reactive response" of astrocytes is not linked exclusively to chronically altered conditions and it occurs also following episodic dysregulations of neural circuit activities, such as the transient, local hyperactivity of neurons that characterizes epileptic seizures. This strengthens the idea that the "astrocyte reactivity" is not a standardized response and its distinct features (and role) depends on the specific context of the different brain disorders.
2. "Astrogliosis" refers, in general, to the change in astrocyte morphology and the upregulation of GFAP linked to pathological conditions. In contrast, "astrocyte activation" refers to an event that occurs in a physiological context, linked, for example, to a change in calcium signal dynamics or other signaling pathways.
The term "astrogliosis" reminds me of the hypertrophy of the astrocytic processes, whereas "astrocyte reactivity" refers more to functional changes. They are not, in my opinion, synonymous terms.

Dr. Colm Cunningham

1. I think of astrogliosis as a property of a population while astrocyte reactivity should refer to the activation status of individual cells (since astrocyte phenotype is heterogeneous and may be different even in cells that are relatively close together). "Reactive" as commonly used, suggests that an astrocyte has taken up a different phenotype (i.e. has 'reacted' to some change in its environment) but also implies a propensity to NOW be more reactive than it was previously. This fits with the idea of primed astrocytes as hyperactive to a secondary stimulus (i.e. they are more reactive).
2. I think of astrogliosis as representing increased numbers and altered morphology while astrocyte activation simply reflects that they have been in some way activated by pathology or change in the milieu - but this is therefore an ambiguous term - unless one knows what has activated them or what the resulting phenotype is the term 'activated' has no specific meaning. Astrocyte reactivity is probably my favourite because it encompasses how 'reactive' they are: including increased responsiveness to a secondary stimulus.

Prof. Marc Freeman

1- I think the phrase astrocyte reactivity can be used in many scenarios. Simply put, I think of it as the set of changes that astrocytes undergo in response to an external stimulus. This is usually studied in the context of "bad" things like injury or disease, and the small things we know about have been lumped in together. For instance, axotomy has been used to study how astrocytes respond to brain trauma. Some of those same responses (e.g. hypertrophy and increased GFAP) also occur in

astrocytes in neurodegenerative disease, but I suspect if we performed a much more comprehensive study (like RNA-seq) of how astrocytes respond to axotomy versus neurodegenerative disease that we would see major differences. So I prefer a looser definition, which can include the spectrum of responses that glia exhibit in response to stressful stimuli.
2- I think astrogliosis implies that there is some proliferation going on, in addition to changes in gene expression or morphology. The same is not necessarily true for the other terms. I think astrocyte activation and reactivity are fairly interchangeable, and fit with the definition described above.

Prof. Elena Galea

1- The so-called reactive astrocytes are astrocytes undergoing a stress response to tissue damage. Such response is accompanied with: (i) striking morphological change caused by thickening of intermediate filaments composed of GFAP, and (ii) context-specific changes in their transcriptomic profiles, for 'reactive' astrocytes are molecularly heterogeneous. As to possible functions, in acute injury, I consider astrocyte reactivity as an adaptive response aimed to isolate the damaged tissue from the healthy one by a scar; the healthy tissue eventually recovers homeostasis with newborn astrocytes from GFAP$^+$ NSC cells. By contrast, in chronic injury astrocyte reactivity might be maladaptive, and render astrocytes chronically dysfunctional with respect to their homeostatic and computational roles.
2. 'Reactive astrocytes' and 'astrogliosis' are equivalent, and they are non-useful terms because they refer to a GFAP-driven morphological change that does not inform about context-specific molecular and functional heterogeneity, and hence it says nothing about the net contribution of such astrocytes to disease pathogenesis. The term 'astrocyte activation' in for physiological contexts, but it might be too vague; if we do not say 'active neurons', we should not say 'active astrocytes' either.

Prof. Vittorio Gallo, PhD

1. Morphological, molecular and functional changes seen in astroglial cells/astrocytes responding to CNS injury and other neurological diseases.
2. No, I don't consider these as synonymous terms. Astrogliosis involves astrocyte proliferation. Activation and reactivity don't necessarily involve proliferation. Astrocyte activation could involve intermediate changes that precede a full reactive phenotype.

Prof. Magdalena Götz

I guess I would be very simplistic and define a reactive astrocyte as an astrocyte reacting to a major alteration in its environment. I would also add that this is not one state but several (and probably many) states. We have shown for example that in APPPS1 mice where astrocytes are very 'activated' (large some, very high GFAP levels) they do not proliferate, while in traumatic and ischemic injuries a subpopulation of them proliferates (see e.g. Sirko et al., 2013, 2015, Frik et al., 2018). So the term reactive astrocytes comprises a) a heterogeneous population and b) different states depending on the alteration in the environment.
In regard to your second question I guess each of these terms is not well defined by themselves, so in that sense it is even more difficult to say whether and how much they are overlapping. In 'colloquial terms', i.e. in many manuscripts and reviews they are used interchangeable, but by definition they are not.
The best suggestion I could come up with is to order them according to the strength/intensity/extend of changes to the homeostatic state – astrocyte activation could be a mild activation, astrocyte reactivity would probably more used for stronger changes and astrogliosis is often used for even stronger stages often accompanied by inflammation. But your review could achieve something very important if you'd suggest more clean definitions.

Prof. Philip G Haydon

Here is a simple overview of my thoughts. Of course, as you realize, this is a complex process and a few sentences leads to oversimplifications. …

1. Reactive astrocytosis is defined and characterized by gross hypertrophy of the cell body and processes of astrocytes in injured and/or diseased areas of the brain. Accompanying these anatomic changes is enhanced expression of, but not limited to, glial cytoskeletal proteins such glial fibrillary acidic protein (GFAP) and vimentin. However, this is not a bistable state - reactive vs non-reactive - but rather there are different shades of grey and significant heterogeneity in astrocyte reactivity.

2- No. I think it is important to clearly define each term being used and use the different terms to discriminate between different states/processes that are on-going in the astrocyte. Too frequently terms are not defined leading to confusion.

Prof. Dr. Helmut Kettenmann

1. *My definition would be an altered phenotype manifested by morphological alterations, change in RNA expression pattern and functional parameters due to an alteration in the brain environment related to pathology or injury. This altered phenotype can be quite diverse depending on the type of pathology.*
2. *I think that these three terms have been used to describe the same event.*

Prof. Schuichi Koizumi

1- I think that the definition of astrocyte reactivity can be divided into two. The first is to distinguish by form and molecular biological difference, the second is from the aspect of function (beneficial & hazardous etc). In either case, I think that it is necessary for astrocyte to become a state as if it became a different types of cells from normal astrocytes.

2- I think that these three words contain the same meaning in part. Astrogliosis means reactive astrocytes, but has a strong implication of becoming a proliferative astrocyte. Astrocyte reactivity and astrocyte activation have almost the same meaning, i.e., they become reactive astrocytes.

Dr. Andras Lakatos

1. For me this term encompasses astrocyte responses to any external stimuli, while historically this has been used to describe morphological changes with increases in intermediate filaments in response to injury. It transpires that people still strongly associate this term with a detrimental astrocytic function that is non-permissive for CNS repair. I don't see the problem of using this term in the right context, but then it should be decorated with adjectives implying the type of pathological or physiological astrocyte state. In the new era of single cell omics it can be further argued if "astrocyte reactivity" should be used as a collective term at all to describe intracellular changes, given the differences in astrocyte responses based on their subpopulation- or region-specific variations in transcription and signalling.

2. I regard astrocyte activation and reactivity as interchangeable terms because both words carry the meaning of "response to any stimuli". However, there is probably more consensus regarding the vast difference between astrocyte activation (reactivity) and the term astrogliosis that latter indicates a cascade of events following activation by pathological stimuli, which may involve at least astrocyte proliferation if not other processes as well, such as activation/influx of various cell types into lesion environments.

Prof. C. Justin Lee

NB: […] edited for confidential information about unpublished data

I fully agree with you on the fact that reactivity is loosely defined in the past and we need to establish a consensus. …
The problem of use of the term, reactive astrocyte is that it is based on the morphological criteria. And traditionally, people have called reactive astrocyte as the one that shows hypertrophy GFAP staining. We have been studying this hypertrophy of astrocytes in depth and we have finally found the molecular mechanism of this hypertrophy (or cause of this hypertrophy). We currently submitted our work, […].

Therefore, my answer to your question #1 is that I would define reactive astrocyte as the one that contain GABA at least in cortical and hippocampal astrocytes. The answer to your question #2 is that the terms "astrogliosis", "astrocyte activation" and "astrocyte reactivity" cannot be synonymous according to my definition of active and reactive astrocytes. Active astrocytes= contain proBDNF with no GABA. Reactive astrocytes = elevated MAO-B, high GABA, low proBDNF. Astrogliosis= severe reactive astrocyte. […]
I don't think the role of reactive astrocytes in neurodegenerative diseases is "elusive." The meaning of reactive astrocyte might be elusive, but the role of these GABA positive reactive astrocytes has been clearly laid out in numerous occasions. For example, in Alzheimer's disease, we and Gong Chen group have clearly demonstrated that the GABA positive reactive astrocytes release GABA to strongly inhibit neighboring neurons to cause learning/memory impairment.
Although not published, we have been trying to publish the similar mechanistic story […]
In summary, the GABA-containing, GABA-releasing, H2O2-producing reactive astrocytes have critical role in neurodegenerative diseases. Unfortunately, most of these works are not published yet and will be available hopefully soon.

Dr. Shane Liddelow

1. *The OED states that 'reactivity' is 'The quality of being reactive or the degree to which something is reactive'. It follows then a 'reactive astrocyte' is any astrocyte that has a FUNCTION that is altered from its normal physiological state, that is, an astrocyte with a function that is altered in response or reaction to some non-physiological setting. There could then be a reactive astrocyte for each individual stimulus (and infinite number of states), or a smaller number of states that collectively respond to a combined number of stimuli.*
2. Yes. Though I would be less inclined to use 'activation' as it suggests that astrocytes are not normally active in a physiological state - and we know this is untrue. MY personal preference is 'reactivity' but 'gliosis' is similarly useful. Again, I think the most important thing is not so much the nomenclature itself, but what functions are changed (though I do have a pet hate for researchers constantly coming up with their own names for things, rather than sticking with names already in the literature, but adding to the body of knowledge that we have)... but all things must change with time.

Prof. Albee Messing

My first thoughts about a definition of a reactive astrocyte go back to Tolstoy:
"All happy families are alike; each unhappy family is unhappy in its own way."
But, trying to be more helpful, I'd say my definition is a "change in properties in response to injury or disease, reflected by alterations of morphology, gene expression, and function." This intentionally excludes anything related to what you might call "normal physiology". And then your second question of course includes several loaded terms that are difficult to pin down. In my mind astrogliosis fits my definition above most closely – an abnormal state. But obviously astrocytes get "activated" in many normal ways, and in English at least "activation" and "reaction" could both easily apply to normal states, so it gets messy very quickly.

I imagine you are contacting me because of Alexander disease. I think the astrocytes here acquire some properties in common with other types of reactive astrocytes, but also have properties that are unique to the disease.

Prof. Keith Murai

1. *IMO, astrocyte reactivity is the conversion or transformation of the basal state of an astrocyte to a dynamic, pathological state that normally involves a constellation of structural and molecular changes including astrocytic process hypertrophy and remodeling, upregulation of particular genes (i.e. classical GFAP increase and neuroinflammatory cytokines), activation of specific signaling pathways (i.e. STATs), and potentially re-instatement of cell division (although this is not always the case). However, astrocyte reactivity can differ between brain regions and is influenced by injury type and likely by astrocyte heterogeneity.*
2. *I consider astrogliosis and astrocyte reactivity as mostly synonymous terms although I tend to think of astrogliosis more in terms of astrocyte reactivity following physical CNS damage (i.e. spinal cord injury) that is accompanied by glial scar formation. Although this may be more related to my past background in spinal cord injury research. I have never used the term 'astrocyte activation'. I reserve the term 'activation' for microglia which has a different time-course and series of cellular changes.*

Prof. Christopher Norris

I'll answer both of these together.
I think astrocyte reactivity, astrocyte activation, and astrogliosis can overlap, but are not the same things.
To me, astrocyte reactivity is an initial (primarily biochemical) response to some form of injury or degeneration. The reaction may vary depending on the area of the brain, the cytoarchitectural context (e.g. gray vs white matter, surrounding neuron subtypes), and perhaps the nature of the insult. For instance, a reactive astrocyte may release trophic factors, or ROs; may adjust its processes around synapses or blood vessels; and/or may adjust the connectivity of its gap junction network.
The distinction between reactive and activated astrocytes is harder to articulate (at least for me). I think that once an astrocyte reacts to an injury it can undergo a phenotypic transformation involving induction of unique transcriptional programs, functional changes, and structural changes (e.g. GFAP induction). So, to me, "astrocyte activation" describes the post-phenotypic transformation of reactive astrocytes (which may also vary in nature depending on area of the brain, context, …etc). Astrocyte activation can be short lived (resolvable) or chronic (unresolved).
I've always thought of astrogliosis as the proliferation of astrocytes, which may persist in an activated state.

Dr. James O'Callaghan

1. *Astrocyte reactivity is the homotypic response of astrocytes to elaborate gene expression and proteins following all types of neural (neuronal and glial) injury, including disease, trauma, and chemically-induced damage. The hallmark remains the enhanced expression of GFAP (both biochemically and morphologically because astrocyte reactivity is characterized by the accumulation of glial filaments at the EM level and GFAP remains the major protein component of these filaments, therefore, by definition, if you accumulate glial filaments as a signature of astrocyte reactivity you will accumulate GFAP at immunohisto and immunoassay level. Yes, you can argue about subtypes of GFAP positive astrocytes based on a variety of gene expression and morphological criteria…but by and large they have to be GFAP positive to be an astrocyte and respond with an increase from all types of damage.*
2. *Yes, as long as these 3 terms refer to responses of astrocytes to CNS injury*

Prof. Seiji Okada

I strongly agree that terminology of astrocyte research has highly ambiguous.
For me, non-native speaker for English, it is difficult to understand or explain subtle nuances, but in my opinion,
1. Astrocyte reactivity is a cellular response to several kinds of environmental changes for the homeostasis maintenance or tissue repair.

2. I consider "astrocyte activation" and "astrocyte reactivity" as almost same, both term are for the cellular state or ability. Activated or reactive astrocytes can revert to the un-activated or normal state. However, astrogliosis is the "process" of glial scar formation. Glial car is a basically paramagnet, stable tenacious tissue to seclude the injury site from healthy tissue. This process is crucial for the tissue repair after the CNS insult (Please refer Okada et al., Nat Med, 2006).

Dr. Stéphane H.R. Oliet

It is a complex question. I am not a specialist on astrocyte reactivity but I think that it does not necessarily imply astrogliosis.
Tome, astroglial reactivity, which has become "astroglial activation" for some researchers because they do not like the "reactive" term, corresponds to a rather radical change in the morphology and biochemistry of astrocytes, which may or may not be accompanied by gliosis.
This change occurs in response to a stress / stimulation, often of pathological type.
But the main mistake would be to think that this reactivity is the only mode of "activation" of astrocytes. A gain or loss of function of these cells can be achieved without reactivity and most of their physiological functions occur in a non-reactive state.

Dr. Aude Panatier

1- Astrocyte reactivity is a modification of the "physiological state" of the astrocyte that will "allow" (or induce) it to integrate and in turn regulate differently the information.
It is going from a modification of gene transcription to a modification of astrocyte morphology, and its functional interaction with, blood vessels, synapses and neighboring cells.
2- For me "astrogliosis" implicates an increase of the astrocytic domain, which is probably not really the case. It can be the ultimate state of "astrocyte reactivity".
So astrogliosis is not always synonymous of astrocyte reactivity.
Regarding "astrocyte activation", for me yes, it can be synonymous of "astrocyte reactivity".

Prof. Luc Pellerin

1. Astrocyte reactivity is closely linked to the notion of phenotypic change. Astrocytes undergo modifications to move from a "resting" state to a "reactive" state. The classical markers of such a phenotypic change are 1) Altered morphology with a thickening of processes 2) enhanced expression of characteristic proteins, GFAP being the gold standard. The appearance of this new phenotype has been observed in several pathological situations and often associated with the notion of inflammation. The presence of cytokines, either produced by reactive astrocytes or acting on astrocytes to induce phenotypic change is characteristic of such an inflammatory state. The notion of proliferation often associated with reactive astrocytes in the past is controversial and contested. Not much is known about the functional consequences of such a phenotypic change although it is likely that it will modify several aspects of their numerous features (e.g. metabolic, trophic, signalling, etc).
2. I do not consider "astrogliosis", "astrocyte activation" and "astrocyte reactivity" completely equivalent. This is particularly true for "astrogliosis".
Astrogliosis contains the notion of proliferation, in addition to reactivity

Astrocyte activation is the passage from resting astrocyte to reactive astrocyte
Astrocyte reactivity is the capacity to become as well as the state of reactive astrocytes

Dr. Gertrudis Perea

1. It would be the physiological changes happening into astrocytes triggered by stimuli of different nature. The main changes are typically related with GFAP expression (extension and thickness of the processes); however, other changes take place in shorter time periods, such as Ca2+ signaling, glutamate/GABA uptake, K+ buffering, gap-junction coupling, which contribute to transiently alter the function of astrocytes over the neuronal circuits.
2- No, I consider that "astrocyte activation" and "astrocyte reactivity" may represent the physiological response to astrocytes to normal (activation) and abnormal (reactivity) neuronal activity, represented by neurotransmitter release, K buffering, pH measurements, glucose/metabolic state, etc.
On the other hand, astrogliosis could be considered as a new physiological and permanent state after neuronal/brain insult.

Prof. Dr. Gabor Petzold

1- Astrocyte reactivity is an umbrella term for the signaling mechanisms employed by astrocytes in response to acute or sustained pathological stimuli. The main component of this response can be molecular, functional or morphological, or a combination of those. Moreover, this astroglial phenotype is not only a reaction to brain pathology, but also an important contributor to neuronal regeneration and survival. Whether reactive astrocytes amplify or ameliorate neurodegeneration likely depends on the nature and duration of the stimulus, as well as the presence of co-morbidities and the contribution of other cell types.
2- Surprisingly, although changes of astrocytes to brain injury have been known for over a century, the terms describing these alterations have remained highly subjective and ill-defined. To me, these terms describe points on a continuous spectrum. "Astrocyte activation" describes any change in astrocyte function, e.g. molecular or functional, without necessarily implying a pathological event or morphological changes. "Astrocyte reactivity" implies that the stimulus is strong enough to perturb brain homoeostasis, evoking a functional and morphological reaction of astrocytes. "Astrogliosis" is the strongest reaction of astrocytes, and implies morphological changes in astrocytes that result in varying degrees of gliotic scar formation.

Dr. Frank W. Pfrieger

1. Reactivity is the ensemble of changes in astrocytes that are evoked by a pathologic condition (genetic, injury etc.)
2. No. Astrogliosis or gliosis is a bit ill-defined, but it may mean the same. Astrocyte activation does not necessarily require a pathology.

Dr. Stefanie Robel

1. My definition for astrocyte reactivity is pretty broad: Any changes to astrocyte morphology, transcriptome, proteome or function. This can be induced by acute injury, neurological disease or aging.
2. I consider astrocyte activation to be different from astrocyte reactivity. Activity to me means that an astrocyte domain or subdomain is functionally active, for example in response to neuronal activity.
I use astrogliosis and astrocyte reactivity synonymous.

Prof. David Rowitch

1- I consider reactive astrocytes to be defined by marker expression (eg, GFAP) in gray and white matter as well as morphology. They can have both damaging and beneficial functions in the context of injury. There in fact might be heterogeneity in RA across regions of the brain, but this has not been sufficiently defined.

2- No, I consider astrogliosis to be a clinical term signifying brain injury. Activation and reactivity could mean more subtle changes seen in response to cytokines or intrinsic cell death of neighboring cells. But, all these terms are rather non-specific and confusing!

Dr. Alberto Serrano-Pozo

"Astrocyte reactivity" = the potential or capacity of astrocytes to mount an astrocyte reaction. Astrocyte reaction (or response) is a change in astrocyte phenotype that is triggered by external noxious stimuli in pathological brain conditions and/or by normal aging, involves the turning on and/or off of specific gene expression programs depending on the trigger, leading to functional changes, and may or not be accompanied by morphological changes and proliferation.

"Astrogliosis", "astrocyte activation" and "astrocyte reactivity" are, in my view, not synonyms and therefore not interchangeable. I believe the term "astrogliosis" should be abandoned because the suffix "-osis" in medicine generally implies a pathological state (i.e. neurosis) and, specifically in pathology, implies proliferation/increased number (i.e. leukocytosis, neurofibromatosis) or invasion/infiltration/spreading (i.e. carcinomatosis, amyloidosis), whereas astrocyte reaction is not necessarily associated with a pathological state (i.e. normal aging) or associated with astrocyte proliferation or migration and invasion. The term "astrocyte activation" suggests that astrocytes can react or "set themselves off" autonomously or for no reason, and fails to recognize that there is usually a trigger of astrocyte reaction (including aging), and that astrocyte reaction may involve inhibition (rather than activation) of some normal functions (i.e. neurotrophic, synaptic). The term 'reactivity" literally means potential or capacity to react, which is different from the "reaction" itself. Therefore, I prefer the terms "astrocyte reaction" or "astrocyte response" to refer to the results or effects of "astrocyte reactivity". The concept of "astrocyte reactivity", devoid of its consequence – the astrocyte reaction -, may fit better with the scenario of normal aging, similar to the concept of "microglia priming".

Prof. Dr. Swetlana Sirko

"astrogliosis", "astrocyte activation" and "astrocyte reactivity" are related but not synonymous terms.

Personally, I prefer to speak about "astrocyte reactivity" as the ability of astroglia to develop an adequate response to a local or systemic pathophysiological stimuli. However, the degree of reactivity (regardless at which level, e.g. transcriptional, morphological, behavioural) is strongly dependent on the type, localization and severity of pathology but also on state of health as well as age of organism.

"astrogliosis" as a state of astroglial population associated with chronic, mostly irreversible changes of astrocyte phenotype in context with neuropathologies.

"astrocyte activation" as a very quick functional adaptation on different physiological (and not necessarily pathological) needs, which can up to a point compensate for natural (or pathological) changes (e.g. activated state of astrocytes in aged brain).

In principe, it is like temporal changes in astrocyte state, starting with astrocyte activation (as result of physiological adaptation or exposure to path stimuli) -> astrocyte reactivity (multiple changes in phenotype at different levels)-> astrogliosis (chronic, mostly irreversible changes of astrocyte phenotype). This is, if you want, like determined in the Newton's third law: the action and the reaction are equivalent in magnitude and simultaneous part of an interaction, neither exists without the other.

Prof. Michael Sofroniew

I would imagine that a definition should be broad and able to encompass different roles and functions. People made have contrasting views about specific things, but the general concept should be inclusive and flexible.

1- 'Astrocyte reactivity' is a spectrum of changes in astrocytes that occur in response to all forms and severities of CNS injury and disease. These changes vary with the nature and severity of the insult along a gradated continuum of progressive alterations in molecular expression, progressive cellular hypertrophy and, in severe cases, proliferation and scar formation. The changes associated with astrocyte reactivity are regulated in a context-specific manner by a diverse set of molecular signaling events. The changes undergone by reactive astrocytes have the potential to alter astrocyte activities both through gain and loss of functions that can impact both beneficially and detrimentally on surrounding neural and non-neural cells. Astrocyte reactivity is not an all-or-none response, nor is it a single uniform process, nor is it ubiquitously synonymous with scar formation. Instead, astrocyte is a finely gradated continuum of progressive changes in gene expression and cellular changes that are subtly regulated by complex inter- and intra-cellular signaling mechanisms.

We first proposed and published these definitions 10 years ago because there were no formal modern definitions in the literature at that time (see full quotes from 2 papers below).
Sofroniew (2009) Trends Neurosci 32:638-647
Sofroniew and Vinters (2010) Acta Neuropathol 119:7-35.
In my opinion, the definitions are still valid and I do not know of any new findings that would compel me to change or update them.
You mention that there is a lot of variety and contrasting views in the definitions that people have sent you. I think that is interesting and perhaps rather sad that after so many years there is still confusion among 'experts'… - - It reminds me about the poem of the blind men who argued about their descriptions of the elephant …

2- I consider "astrogliosis" and "astrocyte reactivity" as synonymous and I use them interchangeably. However, I strongly disagree with the use of the term "astrocyte activation" to refer to 'astrocyte reactivity'. This practice is out of date and is left over from the time when astrocytes in healthy tissue were referred to as "resting". There is no such thing as a "resting" astrocyte. Astrocytes in healthy tissue continually exhibit physiological activation in the form of transient, ligand-evoked elevations in intracellular calcium ($[Ca^{2+}]_i$) that represent a type of astrocyte excitability, involved in mediating many critical dynamic astrocyte functions, including interactions with synapses and regulation of blood flow as discussed in other articles in this volume. Thus, astrocyte activation can range from physiological contexts in healthy CNS to pathophysiologic contexts that involve responding to CNS injury or disease. In keeping with these definitions, there seems to be no place for the notion of an "activated astrocyte" as a single uniform entity that occurs only in response to injury or disease.
(I have published this idea in the article Sofroniew MV (2015) Astrogliosis. Cold Spring Harb Perspect Biol 7:a020420. – the direct quote is below)

Here are some direct quotes from papers:
I hope this all helps with your article.

Direct quote on definition of astrocyte reactivity taken from the article Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32:638-647.

"In spite of the increasing recognition that astrocytes play central roles in normal CNS function and that reactive astrocytes are primary responders to injury and disease, the concept of reactive astrogliosis seems generally elusive, with no commonly agreed upon definition or model. This article proposes a definition and model of 'reactive astrogliosis' that integrates four interdependent

key features: (i) reactive astrogliosis is a spectrum of changes in astrocytes that occur in response to all forms and severities of CNS injury and disease including subtle perturbations; (ii) the changes undergone by reactive astrocytes vary with the nature and severity of the insult along a gradated continuum of progressive alterations in molecular expression, progressive cellular hypertrophy and, in severe cases, proliferation and scar formation (Figs. 1,2), (iii) the changes of astrogliosis are regulated in a context-specific manner by specific signaling events that have the potential to modify both the nature and degree of those changes; (iv) the changes undergone during reactive astrogliosis have the potential to alter astrocyte activities both through gain and loss of functions that can impact both beneficially and detrimentally on surrounding neural and non-neural cells.

According to this definition and model, reactive astrogliosis is not an all-or-none response, nor is it a single uniform process, nor is it ubiquitously synonymous with scar formation. Instead, reactive astrogliosis is a finely gradated continuum of progressive changes in gene expression and cellular changes (Figs. 1,2) that are subtly regulated by complex inter- and intra-cellular signaling as discussed in detail below. In its mild and moderate forms, reactive astrogliosis exhibits the potential for resolution if the triggering mechanism has resolved, in which the cells return to an appearance similar to that in healthy tissue (Fig. 2A). The extent to which various molecular changes resolve or persist is not well known. In this context, it is interesting to note that, in healthy tissue, the extensive network of finely branched processes of individual astrocytes occupy contiguous non-overlapping domains [10]. In mild-to-moderate reactive astrogliosis, there appears to be preservation of the individual non-overlapping domains of reactive astrocytes in spite of the hypertrophy of the cell body and processes [11] (Fig. 1B, 2A). At its extreme level of activation in response to overt tissue damage and inflammation, reactive astrogliosis involves scar formation that incorporates newly proliferated cells and in which astrocyte processes overlap in manners not seen in healthy tissue [12-14] (Fig. 1C,2B). It deserves mention that astrocytes interact with other cell types, in particular fibromeningeal and other glial cells (such as NG2-positive glia) to form complex glial scars in the CNS [14, 15] (Fig. 2B). The structural changes associated with scar formation are long lasting and persist long after the triggering insult may have resolved (Fig. 1C,2B). The striking potential differences along the continuum of reactive astrocyte responses to insults of different kinds are likely to be of importance when considering the functions and impact of reactive astrogliosis on CNS functions, as discussed below. In other words, reactive astrogliosis of different kinds will have different consequences."

Direct quote on definition of astrocyte reactivity taken from the article Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119:7-35.

Based on a large body of observations in experimental animals, a definition of reactive astrogliosis has recently been proposed [226] that encompasses four key features: (i) reactive astrogliosis is a spectrum of potential molecular, cellular and functional changes in astrocytes that occur in response to all forms and severities of CNS injury and disease including subtle perturbations; (ii) the changes undergone by reactive astrocytes vary with severity of the insult along a gradated continuum of progressive alterations in molecular expression, progressive cellular hypertrophy, and in severe cases, proliferation and scar formation, (iii) the changes of reactive astrogliosis are regulated in a context-specific manner by inter- and intra-cellular signaling molecules; (iv) the changes undergone during reactive astrogliosis have the potential to alter astrocyte activities both through gain and loss of functions that can impact both beneficially and detrimentally on surrounding neural and non-neural cells [226]. Here, we propose to apply and extend this definition to identifying different gradations of reactive astrogliosis and glial scar formation that may be encountered in histopathological examinations of human disorders (Figs. 3,4). According to this definition, reactive astrogliosis is not an all-or-none response, nor is it a single uniform process, nor is it ubiquitously synonymous with scar formation. Instead, reactive astrogliosis is a finely gradated continuum of progressive changes in gene expression and cellular changes. Although the increasing severities of reactive astrogliosis transition seamlessly along a continuum, it is convenient for purposes of description and classification to recognize three broad categories:

Mild to moderate reactive astrogliosis. In mild or moderate reactive astrogliosis there is variable up regulation of expression of GFAP and other genes [226], as well as hypertrophy of cell body and processes that can vary in degree but that occurs within the domains of individual astrocytes [257] without substantive intermingling or overlap of processes of neighboring astrocytes or loss of individual domains (Figs. 3b,4b) [226]. There is little or no astrocyte proliferation in mild or moderate reactive astrogliosis, however, the up regulation of GFAP expression in astrocytes that do not express detectable levels of GFAP in healthy tissue (Figs. 3a,4a) can lead to the staining of more cells (Figs. 3b,4b), sometimes giving the false impression of proliferation. Mild or moderate reactive astrogliosis is generally associated with mild non-penetrating and non-contusive trauma, diffuse innate immune activation (viral infections, system bacterial infections) and in areas that are some distance to focal CNS lesions. Because there is little or no reorganization of tissue architecture, if the triggering mechanism is able to resolve, then mild or moderate reactive astrogliosis exhibits the potential for resolution in which the astrocytes return to an appearance similar to that in healthy tissue [226]. The physiological consequences of mild or moderate reactive astrogliosis are not well understood.

Severe diffuse reactive astrogliosis. In severe diffuse reactive astrogliosis there is pronounced up regulation of expression of GFAP and other genes, together with pronounced hypertrophy of cell body and processes, as well as astrocyte proliferation, resulting in considerable extension of processes beyond the previous domains of individual astrocytes. As a result, there is substantive intermingling and overlapping of neighboring astrocyte processes with blurring and disruption of individual astrocyte domains (Figs. 3c,4c). These changes can result in long lasting reorganization of tissue architecture that can extend diffusely over substantive areas without the formation of dense, compact barriers as found in glial scars along borders to necrotic tissue (see below). Severe diffuse reactive astrogliosis is generally found in areas surrounding severe focal lesions, infections or areas responding to chronic neurodegenerative triggers.

Severe reactive astrogliosis with compact glial scar formation. Severe reactive astrogliosis with compact glial scar formation includes changes associated with milder forms, such as pronounced up regulation of GFAP and other genes, and pronounced hypertrophy of cell bodies and processes. In addition, glial scar formation progresses beyond these changes with groups of reactive astrocytes that exhibit pronounced overlapping of reactive astrocyte processes, obliteration of individual astrocyte domains, evidence of substantive astrocyte proliferation and the clear formation of dense, narrow and compact glial scars (Fig. 4d). Recent experimental evidence indicates that these astrocyte scars act as neuroprotective barriers to inflammatory cells and infectious agents, and that they form in particular along borders to severe tissue damage, necrosis, infection or autoimmune-triggered inflammatory infiltration (Fig. 4d) [33, 59, 68, 95, 226, 251]. An important feature of these glial scars is the interaction of reactive astrocytes with other cell types, in particular fibromeningeal and other glial cells (Fig. 4d) [31, 95], and the deposition of a dense collagenous extracellular matrix that contains many molecular cues that inhibit axonal and cellular migration [219]. Triggering insults include penetrating trauma, severe contusive trauma, invasive infections or abscess formation, neoplasm, chronic neurodegeneration, systemically triggered inflammatory challenges. It is noteworthy that the glial scar formation is associated with substantive tissue reorganization and structural changes that are long lasting and persist long after the triggering insult may have resolved.

The findings summarized here show that there are pronounced differences along the continuum of potential responses of reactive astrocyte to insults of different kinds and severities. These differences are likely to be of consequence when considering the functions and impact of reactive astrogliosis on CNS disorders and pathologies.

Direct quote regarding differences between "astrocyte activation" and "astrocyte reactivity" taken from the article Sofroniew MV (2015) Astrogliosis. Cold Spring Harb Perspect Biol 7:a020420.

We will not use "activation" or "activated astrocytes" as terms that refer exclusively to astrocyte responses to injury or disease. Astrocytes in healthy tissue continually exhibit physiological activation in the form of transient, ligand-evoked elevations in intracellular calcium ($[Ca^{2+}]_i$) that represent a

type of astrocyte excitability, involved in mediating many critical dynamic astrocyte functions, including interactions with synapses and regulation of blood flow as discussed in other articles in this volume. Thus, astrocyte activation can range from physiological contexts in healthy CNS to pathophysiologic contexts that involve responding to CNS injury or disease. In keeping with these definitions, there seems to be no place for the notion of an "activated astrocyte" as a single uniform entity.

Prof. Harald Sontheimer

For me reactive astrocytes are:
Astrocytes that have undergone both morphological and functional changes as a result of insult or injury typically with the purpose to seal of the location of an injury or disease. Functional changes typically include a global loss proteins involved in ion and neurotransmitter homeostasis and a change in extracellular matrix produced.

Reactive astrocytes and astrogliosis are certainly analogous. The activated astrocytes could refer to astrocytes that show Ca2+ signaling

Prof. Dr. Christian Steinhauser

I'm in the astrocyte field since many years, but my impression is that the term 'reactive' astrocyte still lacks a clear definition; everyone understands something different by it.

1. would define an 'reactive' astrocyte as an astrocyte with pathologically altered molecular, functional and/or morphological properties. Given that i) the physiological properties of astrocytes significantly vary during development as well as within and across brain areas (e.g. between hippocampus, cortex, thalamus, Griemsmann et al., 2015, Cereb Cortex), ii) the 'reactive state' usually progresses/worsens with ongoing disease (e.g. in epilepsy, see Bedner et al., 2015, Brain) and ii) in different diseases astrocytes are differently affected, it is clear that there must be a high heterogeneity among 'reactive' astrocytes (certainly not just A1 and A2 astrocytes). Curiously, some colleagues also speak of 'reactive' astrocytes in the physiologically aged brain; I find this usage inappropriate (because an aged brain is not necessarily a diseased brain).
2- 'Astrogliosis' and 'astrocyte reactivity' in my view are synonymous terms. 'Astrocyte activation' of course also occurs in physiology (e.g. when Ca2+ transients are evoked through GPCR activation), therefore the latter term should not be used to describe pathologically altered astrocytes.

Prof. Raymond Swanson

1) I do not think the term reactive astrocyte has any uniform meaning, and I try to avoid the term. My understanding is that it was introduced in the 1800s by pathologists (French, I think) who noted enlarged astrocytes near sites of injury or tumors; hence the term "reactive". It was later found astrocytes with this morphology have increased GFAP, and that later became a defining feature. But, an assumption was made that all cells with these hallmarks are similar to one another and share similar other features. That part is almost certainly wrong; e.g. there are no data that I know of that all cells with reactive morphology have the same changes in gene expression, and lots of reasons to think they do not. This is exactly parallel to the microglia, where the term "activated" pertains to any cell with retracted processes despite the proven fact that the actual gene expression patterns of these cells is very diverse.
2) Yes, I consider them all synonyms, and all equally uninformative.

Prof. Alexej Verkhratsky

…indeed in my view we do not have an agreed and proper definition of "reactive astrogliosis";

*My definition (taken from **Verkhratsky A, Zorec R, Parpura V (2017) Stratification of astrocytes in healthy and diseased brain. Brain Pathol 27:629-644 doi:10.1111/bpa.12537**) but this was reiterated in many of my papers in last 3-5 years) is:*

Reactive astrogliosis is an evolutionary conserved defensive reprogramming of astroglia aimed at: (i) increased neuroprotection and trophic support of nervous tissue; (ii) isolation of the lesioned area; (iii) reconstruction of the damaged blood-brain barrier; and (iv) providing for post-lesion regeneration of brain circuits (10, 177, 178, 232). Activation of astrocytes is a complex process which arguably produces multiple "reactive" phenotypes, which demonstrate disease specificity. Gene expression profiling of reactive astrocytes demonstrated significant context-dependent (ischaemia vs. endotoxin activation) differences (290). All in all, initiation of astrogliotic programme proceeds through a controlled continuum of changes in cellular biochemistry and function that are tuned to the nature and strength of the insult.
It is cumbersome and still I believe we may need to expand it even more.

The common definition of astrogliosis as hypertrophy and increase in GFAP/Vim expression is of course trivial and misleading; e.g. GFAP may go up and down in physiological conditions for example in SON in circadian fashion.
The recent addition of A1/A2 is welcome as it shows two broad groups of phenotypic outcomes and yet I feel that the reality is much more complex and ether are many more phenotypes which are context, age, disease, comorbidity &c., &c. specific.
Finally I do not know what to think about "neurotoxic" phenotypes - obviously evolution never selects for anything detrimental unless it increases survivability at large; so neurotoxic forms might also have positive defensive meaning; (in a way similarly to scar formation which is definitely protective but could be detrimental in spinal cord injury but evolution does not care about species with broken spine...).
(….)
yes "astrocyte activation", "astrocyte reactivity" and "astrogliosis" refer to the same process; you also forgot another word frequently used: "astrocytosis"

Prof. Andrea Volterra

1. I don't think we have a defined understanding of what a reactive astrocyte is. Previous work indicated that a major feature of reactive astrocytes was their change in morphology, notably the presence of thicker processes, but this was then understood to be an artefact of GFAP (cytoskeletal) staining. Today reactivity is thought to drive a more global "program" change, such as the return to a more immature and plastic state of the astrocyte, accompanied by a large change in its gene expression profile. Since it is increasingly evident that astrocytes are anatomically and biochemically heterogeneous depending on brain areas and circuits, I suspect gene expression changes related to reactivity will depend also on the specific molecular fingerprint of different astrocytic subpopulations. Likewise, they will depend on the specificities of the local microenvironment surrounding an astrocytic population. Moreover, the events triggering reactivity maybe quite different, from microorganism colonization of brain parenchyma, to vascular accidents, to acute traumatic insults to slow processes of protein aggregation and deposition, etc. I suspect astrocytes will turn out to react differently to any of such different conditions. In synthesis, the combination of the intrinsic features of a given astrocytic population, of its microenvironment and the type of insult will concur to produce different types of reactivity and different functional outcomes.
2. I do not personally consider "astrogliosis", "astrocyte activation" and "astrocyte reactivity" as synonymous terms. However, we should have a precise definition for each of these phenomena and I don't think we have. So, this leads many authors to interchange them.

Dr. Ina Wanner

Astrocyte reactivity *is the active response of viable astrocytes to mechanical injury, non-neural cell contact or a chemical insult in order to protect CNS tissues adjacent to injuries and launch a systemic immune response. Viable astrocytes undergo reactivity, not wounded or dying astrocytes that release diagnostic biomarkers due to integrity compromise [1, 2]. Reactive astrocytes activate signaling pathways like the JAK-STAT cascade that induce gene expression changes. Results are proliferation, functional and structural astrocyte changes that contribute to the formation of a scar and wound healing [3]. Astrocyte shape changes after traumatic CNS injuries or after mechanical trauma or non-neural cell confrontation in vitro are defined by hypertrophy – displaying thick and elongated processes [4-7]. Injury models show a stereotypical astrocyte choreography of aligned elongated processes that wind around microglia or meningeal fibroblasts in co-cultures and that form borders around tears from mechanical injury inflicted by pressure-pulse stretching [4, 5, 7]. Perilesional reactive astrocytes in the injured spinal cord are elongated and associate with each other to bundles that corral non-neural cells and prevent their infiltration. This astrocyte-astrocyte interaction forms a scaffold that establishes the scar border around the lesion limiting its expansion [3, 7].*

Astrocyte activation *during scar formation is captured by astrocyte-specific protein synthesis after mouse spinal cord injury [8]. Major changes were in protein groups of the biological processes, the JAK-STAT cascade, inflammation, NO biosynthesis, calcium ion transport, gliogenesis and angiogenesis; and of the cellular components, cell adhesion, vesicle and extracellular matrix. These essential protein groups mediate scar assembly and are aimed at re-establishing the neuro-vascular unit and ion homeostasis. Interestingly, quantitative proteomic analysis of in vitro matured human trauma-reactive neocortical astrocytes resulted in changes of the same essential protein groups [manuscript in preparation, [5]. These findings are supported by literature analysis grouping most frequently reported astrocyte protein changes after spinal cord injury in a survey of over 200 publications in the last 30 years.*

Astrogliosis, astrocyte reactivity and activation *are essentially describing similar astroglial processes. In the literature the term "astrogliosis" associates more frequently with histopathology, while the term "astrocyte activation" associates more frequently with expression changes. The term "astrocyte reactivity" is applied in vivo and in vitro models of astrocyte injury responses. Thus, their heritage and common use differ, yet since structural changes are based on molecular and protein changes, these terms can be seen as part of the same astrocyte response program.*

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Supplemental Table 1

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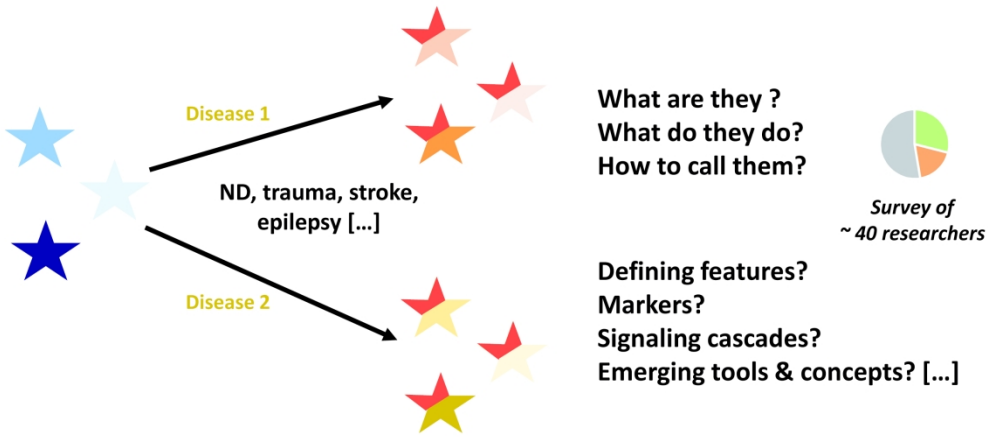

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