

Reactive astrocyte nomenclature, definitions, and future directions

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209 **Abstract**

210
211 Reactive astrocytes are astrocytes undergoing morphological, molecular, and functional
212 remodelling in response to injury, disease, or infection of the central nervous system (CNS).
213 Although this remodelling was first described over a century ago, uncertainties and controversies
214 remain, regarding the contribution of reactive astrocytes to CNS diseases, repair, and ageing. It is
215 also unclear whether fixed categories of reactive astrocytes exist, and if so, how to identify them.
216 We point out the shortcomings of binary divisions of reactive astrocytes into good/bad,
217 neurotoxic/neuroprotective or A1/A2. We advocate, instead, that research on reactive astrocytes
218 include assessment of multiple molecular and functional parameters, preferably in vivo,
219 multivariate statistics, and determination of impact on pathological hallmarks in relevant models.
220 These guidelines may spur the discovery of astrocyte-based biomarkers, and astrocyte-targeting
221 therapies that abrogate detrimental actions of reactive astrocytes, potentiate their neuro- and glio-
222 protective actions, and restore or augment their homeostatic, modulatory, and defensive functions.
223

224 1. Introduction

225
226 ‘Neuroglia’ or ‘glia’ are collective terms describing cells of neuroepithelial
227 (oligodendrocytes, astrocytes, oligodendrocyte progenitor cells, ependymal cells), neural crest
228 (peripheral glia), and myeloid (microglia) origin. Changes in neuroglia associated with diseases of
229 the central nervous system (CNS) have been noted, characterised, and conceptualised from the very
230 dawn of neuroglial research. Rudolf Virchow, in a lecture to students and medical doctors in 1858,
231 stressed that “*this very interstitial tissue [i.e. neuroglia] of the brain and spinal marrow is one of*
232 *the most frequent seats of morbid change...*”¹ Changes in the shape, size, or number of glial cells
233 in various pathological contexts have been frequently described by prominent neuroanatomists.² In
234 particular, hypertrophy of astrocytes was recognised very early as an almost universal sign of CNS
235 pathology;³ “*The protoplasmic glia elements [i.e. astrocytes] are really the elements which exhibit*
236 *a morbid hypertrophy in pathological conditions*”.³ Neuroglial proliferation was thought to
237 accompany CNS lesions, leading to early suggestions that proliferating glia fully replaced damaged
238 neuronal elements.⁴ Thus, a historical consensus was formed that changes in “*the appearance of*
239 *neuroglia serves as a delicate indicator of the action of noxious influences upon the central nervous*
240 *system*”, and the concept of “*reactionary change or gliosis*” was accepted.⁵ While the origin of
241 “gliosis” is unclear (“glia + osis” in Greek means “glial condition or process”; in Latin the suffix
242 “-osis” acquired the additional meaning of “disease”; hence astrogliosis may also carry a
243 connotation of “glial disorder”), the term became universally adopted to denote astrocytic
244 remodelling in response to pathologic conditions. The role of reactive astrocytes in forming a scar-
245 border to seal the nervous tissue against penetrating lesions was recognised, with distinct stages
246 being visualised.⁵ In the 21st century, astrocytes are increasingly viewed as having a critical
247 contribution to neurological disorders. Research into the roles of astrocytes in neurology and
248 psychiatry is accelerating and drawing in increasing numbers of researchers. This rapid expansion
249 has exposed a pressing need for unifying nomenclature and refining of concepts.⁶ Here, we start by
250 providing a working consensus on nomenclature and definitions, and by critically evaluating
251 widely used markers of reactive astrocytes. Then, we describe the advances, and we take position
252 on controversies, regarding the impact of astrocytes in CNS diseases and ageing. Finally, we
253 discuss the need for new names to grasp astrocyte heterogeneity, and we outline a systematic
254 approach to unravelling the contribution of astrocytes to disorders of the CNS. This article is
255 expected to inform clinical thinking and research on astrocytes, and to promote the development
256 of astrocyte-based biomarkers and therapies.

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258

259 2. Too many names

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261 “Astrocytosis”, “astrogliosis”, “reactive gliosis”, “astrocyte activation”, “astrocyte reactivity”,
262 “astrocyte re-activation”, and “astrocyte reaction” have been all used to describe astrocyte
263 responses to abnormal events in the CNS, including neurodegenerative and demyelinating diseases,
264 epilepsy, trauma, ischemia, infection, and cancer. We suggest “reactive astrogliosis” to define the
265 process whereby, in response to pathology, astrocytes engage in molecularly defined programs
266 involving changes in transcriptional regulation, as well as biochemical, morphological, metabolic,
267 and physiological remodelling, which ultimately result in gain of new function(s) or loss or
268 upregulation of homeostatic ones. Although for some researchers, particularly neuropathologists,
269 “reactive astrogliosis” is invariably associated with irreversible changes such as astrocyte
270 proliferation, scar-border formation, and immune-cell recruitment,⁶ these phenomena mainly occur
271 when there is disruption of the blood-brain barrier (Fig. 1a).⁷ We also support the term “astrocyte
272 reactivity” as being broadly equivalent to “reactive astrogliosis”, but emphasizing the capacity of
273 astrocytes to adopt distinct state(s) in response to diverse pathologies. Therefore, “reactive
274 astrocytes”, referring to the cells undergoing this remodelling, is an umbrella term encompassing

275 multiple potential states. We define “state” as a transient or long-lasting astrocyte condition
276 characterized by a specific molecular profile, functions, and distinct impact on diseases, while its
277 “phenotype” is the measurable outcome of that state. Importantly, the changes in astrocytes in
278 response to pathological stimuli are not to be confused with the plasticity of healthy astrocytes,
279 which are constantly being activated by physiological signals in the CNS. For this reason, although
280 transitions from physiology to pathology are progressive and sometimes difficult to define,
281 “astrocyte activation” should be reserved for physiological conditions and not used in pathological
282 contexts, which should be referred to as “astrocyte reactivity”.

283
284 The pathological contexts in which astrocyte reactivity occurs can markedly vary, and may be
285 sporadic or genetically mediated, acute or chronic, due to a systemic pathology (e.g., sepsis),
286 specific injury or disease of the CNS, or a deleterious experimental manipulation. By definition,
287 astrocyte reactivity is secondary to an extrinsic signal, may evolve with time, and, in many
288 situations, is reversible. Astrocytes may also exhibit cell-autonomous disturbances,⁸ as happens in
289 astrocytopathies resulting from mutated alleles of astrocytic genes (e.g. *GFAP* in Alexander
290 disease),⁹ as well as from direct viral infections or exposure to toxic substances that specifically
291 damage astrocytes (e.g., ammonium in hepatic encephalopathy).¹⁰ These astrocytes can be
292 considered “diseased astrocytes” that unequivocally initiate the diseases and may secondarily
293 acquire a reactive phenotype with a distinct impact on disease progression. Mutations in
294 ubiquitously-expressed genes, as in familial neurodegenerative disorders (e.g. Huntington’s
295 disease, HD), or disease-risk polymorphisms in genes highly expressed in astrocytes (e.g., *APOE*
296 in Alzheimer’s disease, AD),¹¹ may also lead to dysfunctional astrocytes that, without being the
297 sole or primary initiators of pathology, may adversely affect outcomes. Terminology
298 recommendations and caveats are summarized in Box 1 and in section 7, below.

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300

301 **3. GFAP as a marker**

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303 Glial fibrillary acidic protein (GFAP)—a major protein constituent of astrocyte intermediate
304 filaments—is the most widely used marker of reactive astrocytes (Table 1).¹² Indeed, up-regulation
305 of GFAP mRNA and protein, as shown with multiple techniques including quantitative PCR
306 (qPCR), RNA sequencing (RNAseq), *in situ* hybridization, electron microscopy, and
307 immunostaining (Fig. 1a, d), is a prominent feature of many, but not necessarily all, reactive
308 astrocytes: (i) increased GFAP content occurs across diverse types of CNS disorders, (ii) is an early
309 response to injury, and, moreover (iii) is a sensitive indicator, detectable even in the absence of
310 overt neuronal death (e.g., when there is synapse loss, minor demyelination, and extracellular
311 amyloid- β oligomers). However, while the degree of GFAP up-regulation in reactive astrocytes
312 often parallels the severity of the injury,⁶ this correlation is not always proportional, perhaps due
313 to regional differences of astrocytes, including basal GFAP content.^{13, 14} In the healthy mouse brain,
314 hippocampal astrocytes have a higher GFAP content than cortical, thalamic, or striatal astrocytes;
315 this, however, does not make hippocampal astrocytes more reactive. GFAP is also expressed by
316 progenitor cells¹⁵ and its expression depends on developmental stages.^{16, 17} In addition, GFAP
317 immunoreactivity has been reported to decrease in a subpopulation of astrocytes in mouse cortex
318 following repetitive trauma,⁶ and in the spinal cord of a mouse model of amyotrophic lateral
319 sclerosis (ALS), probably due to cleavage of GFAP by caspase 3.¹⁸ Expression of *GFAP* is also
320 modulated by physiological stimuli such as physical activity,¹⁹ exposure to enriched
321 environments,¹⁹ and glucocorticoids,²⁰ and it fluctuates with circadian rhythms in the
322 suprachiasmatic nucleus.²¹ Therefore, changes in *GFAP* expression may also reflect physiological
323 adaptive plasticity rather than being simply a reactive response to pathological stimuli. A common
324 mistake is to interpret higher numbers of GFAP-positive cells as local recruitment or proliferation
325 of astrocytes. We recommend to use markers of proliferation (Ki67, PCNA and BrdU

326 incorporation, Table 2), and to combine GFAP immunostaining with other ubiquitous astrocyte
327 markers such as aldehyde dehydrogenase 1 L1 (ALDH1L1), glutamine synthetase (GS), and
328 aldolase C (ALDOC) to correctly estimate astrocyte numbers,²² provided that their expression is
329 stable. Finally, there are discrepancies between observed mRNA and protein levels, perhaps due to
330 differential regulation of translation, post-translational modifications, protein half-life, and
331 antibody epitope accessibility. Overall, although an increase in GFAP content is a strong indication
332 of reactive-astrocyte remodelling, it is not an absolute marker of reactivity, nor does it strictly
333 correlate with the extent thereof, or indicate altered functions of reactive astrocytes.

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336 **4. Morphology revisited**

337

338 Increased GFAP immunoreactivity largely reflects changes in the astrocytic cytoskeleton and tends
339 to exaggerate the degree of hypertrophy, because, with the exception of scar-border astrocytes, the
340 volume accessed by reactive astrocytes does not change, since they remain in their territorial
341 domains.²³ In other words, cytoskeletal reorganization does not necessarily equal astrocyte
342 hypertrophy. Immunohistochemical staining for cytosolic enzymes such as ALDH1L1, ALDOC,
343 GS, and S100B allow the visualization of the somata and proximal processes of astrocytes,
344 although, like GFAP, these markers fail to reveal small processes. Membrane proteins such as the
345 glutamate transporters EAAT1 and 2 are not optimal to assess complex astrocyte morphology, as
346 they tend to produce widespread and diffuse staining.²⁴ In addition, the expression of some of these
347 proteins may change in reactive astrocytes (²², Table 1) and some might be expressed by other cell
348 types in specific brain regions.¹³ Animal models expressing fluorescent proteins in the astrocyte
349 cytosol or membrane through astrocyte-specific transgenesis, or gene transfer with viral vectors,²⁵
350 circumvent the limitations of immunohistochemical analysis. Further, dye-filling methods can be
351 used to visualize whole astrocytes in mice²³, as well as in human brain samples from surgical
352 resections (Fig. 1b).²⁴ Thorough visualisation is necessary because astrocytes undergo distinct
353 morphological changes other than hypertrophy in pathological contexts, including elongation,
354 process extension towards injury site, and some 3D domain overlap.²⁶ In addition, although
355 astrocytes appear to be more resistant than neurons to degeneration and death, loss of primary and
356 secondary astrocyte branches has been reported in mouse models of AD²⁷ and ALS,¹⁸ and in
357 patients with multiple sclerosis (MS).²⁸ Detailed analyses of astrocyte arborization in CNS diseases
358 and injuries are however pending, given that the fine perisynaptic and perivascular astrocytic
359 processes can only be revealed with super-resolution, expansion, or electron microscopy. Finally,
360 clasmatodendrosis (From Greek “klasma”, fragment + “dendron”, tree + “osis”, condition or
361 process) is a form of astrodegeneration characterized by an extreme fragmentation or beading and
362 disappearance of distal fine processes, along with swelling and vacuolation of the cell body. It is
363 observed in neuropathological specimens after severe trauma and ischemia, and in the aged brain.²⁹
364 However, although astrocytes may suffer plasma membrane disruption due to mechanical damage
365 and cleavage of membrane proteins and cytoskeletal proteins including GFAP by proteases in acute
366 brain trauma,^{30, 31} the phenomenon of clasmatodendrosis should be approached with caution,
367 because it may be an artefact derived from *post-mortem* autolysis with no pathophysiological
368 bearing, as suggested by Cajal.³² In summary, GFAP upregulation and hypertrophy are useful, but
369 insufficient markers of astrocyte reactivity that need to be complemented by additional markers
370 (Table 1, Box 1).

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373 **5. Impact in CNS diseases**

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375 Research on astrocytes in CNS diseases has advanced in the last century in line with conceptual
376 and technological progress in astrocyte biology. New approaches have been progressively

377 integrated with existing ones and these continue to evolve. At present, research in reactive
378 astrocytes is an interdisciplinary endeavour combining -omics approaches with physiology and
379 genetic manipulation. Below, we summarize advances and controversies with regards to the impact
380 of astrocytes in CNS diseases from a historical perspective, punctuated by technical advances.

381

382 *From morphology to functional studies*

383

384 From the early 20th century up to the 1980s, the morphological appearance of astrocytes was the
385 only readout of their role in neuropathology. Hypertrophy and increased GFAP content were
386 generally regarded as reflections of a detrimental astrocyte phenotype. The advent of genetic
387 engineering in the early 1990s opened a new phase of research based on astrocyte-targeted
388 manipulation of gene expression. For example, depletion or over-expression of receptors,
389 membrane proteins,^{33,34} cytoskeleton proteins,³⁵ acute-phase proteins,³⁶ heat-shock proteins,³⁷ and
390 transcription factors³⁸⁻⁴⁰ in astrocytes or ablation of proliferative scar-border forming astrocytes,⁴¹
391 was reported to modify (protect or exacerbate) the course of neurological diseases in mouse
392 models. An important conclusion drawn from these studies is that the morphological appearance
393 of astrocytes does not correlate with functional phenotypes, or with their impact on other cell types.
394 Moreover, the overall impact of reactive astrocytes on each disease is complex. For example, the
395 manipulation of reactive astrocytes has resulted in improved,^{38, 42, 43} worsen³⁵ outcomes, and no
396 change⁴⁴ in mouse models of AD and MS.^{40, 45, 46} Plausibly, such differences arise from several
397 scenarios: (i) pathways that ultimately exacerbate, attenuate, or have no impact on ongoing
398 pathology occur in the same astrocyte, such that the selective manipulation of one pathway may
399 mask, or secondarily impact, the manifestation of others, (ii) coexisting astrocyte subpopulations
400 may have opposing effects on pathology,⁴⁵ (iii) in neurodegenerative diseases, a spectrum of
401 reactive-astrocyte phenotypes conceivably coexist in the same brain at a given time point because
402 of the asynchronous progression of neuropathology in different brain regions, (iv) the pathological
403 impact of astrocytes is stage-dependent, as shown in mouse models of MS.^{40,45,46} Finally, pathways
404 inducing astrocyte reactivity may be beneficial in one disease and detrimental in another. For
405 example, activation of STAT3-dependent transcription is beneficial in neonatal white matter
406 injury,⁴⁷ traumatic brain injury,³⁰ spinal cord injury,^{48,49} and motor neuron injury⁵⁰ but detrimental
407 in AD models.^{42, 43} That is, STAT3-mediated transcriptional programs may contribute to
408 malfunctioning astrocyte states in AD models, and to resilient states in other conditions. We broadly
409 define astrocyte resilience as the set of successful astroprotective responses that maintain cell-
410 intrinsic homeostatic functions in neural circuits (Table 2), while promoting both neuronal and
411 astrocyte survival. Lastly, responses of reactive astrocytes may be maladaptive and result in
412 malfunctioning astrocytes, which, in addition to losing homeostatic functions, may also gain
413 detrimental functions, thus exacerbating ongoing pathology.⁶ Numerous mixed scenarios of
414 malfunctioning and resilient astrocytes plausibly exist, with multidirectional transitions among
415 them.

416 Research in the last decade has begun to unravel specific functional alterations in reactive
417 astrocytes underlying complex phenotypic changes. In normal conditions, astrocyte Ca²⁺-based
418 responses, and downstream signalling via neuroactive mediators, exert multifarious effects on
419 synaptic function and plasticity, neural-network oscillations, and, ultimately, on behaviour.^{51,52} In
420 pathology, various functional changes emerge. Astrocyte Ca²⁺ dynamics and network responses
421 become aberrant in mouse models of HD,⁵³ AD,⁵⁴ and ALS,⁵⁵ possibly contributing to cognitive
422 impairment and neuropathology.^{43, 53, 56} Reactive microglia may shift astrocyte signalling from
423 physiological to pathological by increasing production of tumour necrosis factor α , thus altering
424 synaptic functions and behaviour.⁵⁷ Functions lost or altered in reactive astrocytes include
425 neurotransmitter and ion buffering in mouse HD models,⁵⁸ communication via gap junctions in the
426 sclerotic hippocampus of epileptic patients,⁵⁹ phagocytic clearance of dystrophic neurites,⁶⁰ and

427 metabolic coupling by glycolysis-derived D-serine⁶¹ and lactate⁶² in mouse AD models. The
428 excessive release of GABA by reactive astrocytes in AD⁶³ and Parkinson's disease⁶⁴ may be a case
429 of gain of detrimental function. Another example may be the so-called astrocyte neurotoxicity, but
430 we recommend using this term only when increased neuronal death is due to the verified release of
431 an identified toxic factor by reactive astrocytes, and not merely due to loss of trophic or antioxidant
432 support from astrocytes. An example is neuronal damage due to nitrosative stress caused by
433 astrocyte-derived nitric oxide in MS.³³ Finally, a classical gain of beneficial function is the
434 restriction of immune cell infiltration in open injuries by scar-border forming reactive astrocytes.⁷

435 *Transcriptomics and A1/A2 classification*

436
437 Transcriptomics has contributed to a fundamental discovery: astrocytes in the healthy brain are
438 diverse and specialized to perform specific roles in distinct CNS circuits.^{14, 65} Astrocyte diversity
439 in healthy tissue arises from embryonic patterning programs or local neuronal cues.¹⁴ Likewise,
440 reactive astrocytes are also diverse, as unequivocally demonstrated by microarray-based⁶⁶⁻⁶⁸ and
441 RNAseq-based^{48, 69-71} transcriptomic profiling of mouse bulk astrocytes,^{48, 66-70} or of astrocyte
442 populations pre-selected according to cell-surface markers.⁷¹ Such transcriptomic profiling
443 specifically shows that reactive astrocytes adopt distinct molecular states in different disease
444 models,^{48, 66-70} CNS regions,⁷⁰ and in brain tumours.⁷¹ These studies also suggested complex
445 functional changes in reactive astrocytes, including novel regenerative functions,⁷⁰ proliferation,
446 and neural stem cell potential,⁶⁸ as well as loss of homeostatic functions.⁶⁶ They have also identified
447 drug candidates to establish the impact of altered astrocytic pathways in mouse models.^{68, 70}
448 Whether baseline astrocyte heterogeneity influences astrocyte reactivity is an outstanding question.
449

450 In one early transcriptome study⁶⁶ and its follow-up,⁷² it was proposed that mouse astrocytes
451 adopted an “A1” neurotoxic phenotype after exposure to specific cytokines secreted by microglia
452 exposed to lipopolysaccharide (LPS), whereas they acquire an “A2” neuroprotective phenotype
453 after ischemic stroke—two acute pathological conditions. Two correlative signatures of 12 genes
454 with 14 pan reactive genes were proposed as fingerprints identifying these phenotypes and, for A1
455 astrocytes, combined with thorough functional analyses *in vitro*.⁷² Although the A1 and A2
456 phenotypes were not proposed to be universal or all-encompassing, they became widely
457 misinterpreted as evidence for a binary polarization of reactive astrocytes in either “neurotoxic” or
458 neuroprotective states, which could be readily identified in any CNS disease, acute or chronic, by
459 their correlative marker genes in a manner similar to the once popular, but now discarded,
460 “Th1/Th2 lymphocyte and “M1/M2” microglia polarization theories.⁷³ For multiple reasons, we
461 now collectively recommend moving beyond the “A1/A2” labels and the misuse of their marker
462 genes. Importantly, only a subset, often a mix of “A1” and “A2” or pan-reactive transcripts, are
463 upregulated in astrocytes from human HD⁷⁴ and AD^{75, 76} brains, or from several mouse models of
464 acute injuries and chronic diseases of the CNS.^{42, 69, 76, 77} Moreover, the functions of these genes are
465 not known, for, to date, no experimental evidence has causally linked any of the proposed marker
466 genes of “A1” or “A2” astrocytes to either “toxic” or “protective” functions. Thus, the mere
467 expression of some, or even all these marker genes, does not prove the presence of functions that
468 these genes have not been demonstrated to exert. Specifically, complement factor 3 (C3) should
469 not be regarded as a single and definitive marker that unequivocally labels astrocytes with a net
470 detrimental effect. In addition, steadily increasing evidence indicates that any binary polarization
471 of reactive astrocytes falls short of capturing their phenotypic diversity across disorders. For
472 example, single cell/nucleus RNAseq (sc/snRNAseq) studies in mouse models and human brains
473 of chronic neurodegenerative diseases have unravelled numerous stage-dependent transcriptomic
474 states in HD,⁷⁴ AD,^{75, 78} and MS⁴⁰, that do not clearly comply with A1/A2 profiles. In addition,
475 advanced statistics using multi-dimensional data and co-clustering approaches reveals that the
476 “A1” and “A2” transcriptomes represent only two out of many potential astrocyte transcriptomes

477 segregating along several latent variables.⁷⁹ The analyses also indicate that multidimensional data
478 are necessary to establish the distinctiveness of astrocyte phenotypes (Fig. 2). Characterization of
479 the potentially extensive and subtle functional diversity of reactive astrocytes suggested by
480 transcriptomic data is an important future goal.

481

482 *Human stem cells*

483

484 Advances in human induced pluripotent stem cell (hiPSC) technology are being adapted to
485 astrocyte research. Interestingly, astrocytes generated from hiPSC derived from fibroblasts
486 obtained from patients with CNS diseases (usually with a genetic mutation causative of disease or
487 a risk polymorphism) show pathological phenotypes, including dysregulation of lipid
488 metabolism,¹¹ alteration in the contents of the extracellular vesicles released by astrocytes,⁸⁰
489 reduced autophagy,⁸¹ or altered STAT3 signalling.⁸² hiPSC-derived astrocytes are also amenable
490 to study responses to viral infection⁸³ and to specific stimuli.⁸⁴ Nevertheless, caution is in order,
491 for more research is needed to establish hiPSC-derived astrocytes as *bona fide* models of human
492 astrocytes and to determine whether they recapitulate the maturity as well as the temporal, regional,
493 and subject heterogeneity of *in vivo* astrocytes. Importantly, not only are these cells removed from
494 their original milieu, but the serum pervasively used in culture media may render them reactive.⁸⁴
495 In addition, generation of astrocytes from neural stem cells is inherently difficult, and derivation
496 and culture conditions have not yet been standardized, leading to diversity of clone phenotypes.
497 Finally, ageing-related neurodegenerative diseases should be modelled with astrocytes derived
498 from cells from aged subjects, but, in this case, the epigenetic rejuvenation intrinsic to the
499 reprogramming of adult cells arises as a confounding factor to be controlled for.

500

501

502 **6. Are ageing astrocytes reactive or senescent?**

503

504 Healthy brain ageing is not pathological and may be defined as an adaptive evolution of global cell
505 physiology over time.⁸⁵ Aged human brains display only mild and heterogeneous changes in
506 astrocyte morphology or GFAP levels.⁸⁶ Studies in rodents document region-dependent, often
507 contradictory changes in ageing astrocytes, such as an increase in cellular volume and overlap of
508 astrocyte processes, but also atrophy, increase in GFAP content, or even a reduction in the number
509 of GFAP and GS-positive astrocytes.⁸⁷⁻⁸⁹ Notably, ageing is also associated with pronounced
510 regional differences in astrocyte gene expression in mouse brains.^{90,91} However, only a few studies
511 have directly assessed astrocyte functions in the ageing mouse brain.^{85,92} Thus, although the data
512 suggest complex changes in ageing astrocytes, the evidence is not yet sufficient to qualify
513 astrocytes as being *bona fide* reactive during physiological ageing. Nonetheless, with advanced
514 age, cumulative exposure to pathological stimuli may render some astrocytes reactive. To test this
515 hypothesis, a systematic investigation of the molecular properties of ageing astrocytes across
516 different CNS regions in humans, and comparison of physiologically aged and reactive astrocytes
517 in various pathological conditions, is needed, together with functional validations in mouse models.
518 Finally, we suggest caution about extending the concept of senescence to astrocytes based upon
519 the expression of cell senescence markers p16^{INK4A}, increased β -galactosidase activity, and
520 secretion of cytokines,⁹³ because the core definition of senescence (i.e., irreversible cell-cycle arrest
521 in proliferative cells) may not apply to astrocytes, which are essentially post-mitotic cells that rarely
522 divide in healthy tissue. Molecular and functional profiling of putative senescent astrocytes in
523 different diseases is needed to clarify the meaning of p16^{INK4A} expression in post-mitotic astrocytes,
524 as well as the interplay between senescence-like features, reactivity, and ageing in astrocytes.

525

526

527 **7. Are new names needed?**

528
529 Arguably, new names are needed to capture the variety of reactive astrocytes, but current
530 knowledge does not yet allow the objective categorizing of reactive astrocytes. Indeed, the
531 existence of fixed categories defined by molecular and functional features consistently observed in
532 different disease contexts is not yet certain. Nonetheless, two new names have recently been coined
533 to describe the extremes of six astrocytic transcriptional clusters detected by snRNAseq in the
534 hippocampus of AD transgenic and wild-type mice.⁷⁸ In this study, “homeostatic astrocytes” were
535 predominant in healthy mice, whereas “disease-associated astrocytes” were unique to AD mice.
536 We do not support generalization of this “disease-associated” classification to other conditions
537 because only one disease was studied. In addition, the term “homeostatic astrocytes” implies the
538 unproven assumption that other transcriptional astrocyte clusters are dyshomeostatic, while they
539 may be successful homeostasis-preserving adaptations to disease.

540
541 We stress that the expression in full or in part of a pre-determined correlative signature of molecular
542 markers is not, on its own, sufficient to define a functional phenotype of reactive astrocyte. In
543 addition, vague and binary terms such as “neuroprotective” or “neurotoxic” are best avoided in
544 describing astrocyte phenotypes as they are too simplistic to be meaningful, unless they are
545 supported by specific molecular mechanisms, and direct causative experimental evidence. Future
546 classification of reactive astrocytes should, instead, consider multiple criteria including
547 transcriptome, proteome, morphology, and specific cellular functions (Table 2), together with
548 demonstrated impact on pathological hallmarks (Fig. 2).

549
550 For now, we recommend “reactive astrocytes” as the general term for astrocytes observed in
551 pathological conditions (Box 1). The term “injured/wounded astrocytes” should be reserved for
552 astrocytes with unequivocal morphological signs of damage (e.g., beaded processes), as observed
553 in ischemia and trauma.^{30, 31} Descriptions based on misleading generalizations of functional
554 changes and over-interpretation of correlative data should be avoided. We call for a clear
555 operational terminology that includes information about morphology (e.g. hypertrophic, atrophic),
556 molecular markers (Table 1), functional readouts (Table 2), as well as brain region, disease, disease
557 stage, sex, species, and any other relevant source of heterogeneity (Fig. 2). Indeed, the goal is to
558 go beyond the mere categorization of reactive astrocytes, and identify the key variables driving
559 specific reactive astrocyte states, phenotypes, and functions in specific contexts. When addressing
560 similar issues for neurons, scientists are not concerned about categorizing disease-associated
561 neurons into simple generalizable subtypes; rather, the emphasis is placed on understanding
562 specific changes of defined neuronal populations in specific diseases. This principle should also
563 apply to astrocytes.

564

565

566 **8. Towards astrocyte-targeting therapies**

567

568 One goal of research on reactive astrocytes is to develop astrocyte-targeting therapies for CNS
569 diseases. Two challenges preclude translating the wealth of functional and molecular data
570 described in the previous sections into therapies. First, there is a need to unequivocally clarify
571 whether or not reactive astrocytes and their associated signalling pathways significantly contribute
572 to the pathogenesis of specific CNS diseases. The approach should be reciprocal, such that human
573 data inform experimental manipulations in animal models, and animal data are validated in human
574 materials. The second challenge is to develop astrocyte therapies tailored to specific disease
575 contexts. Specific research directions include:

576

577 *Heterogeneity characterization*

578

579 To define astrocyte phenotypes, all sources of heterogeneity should be considered and integrated
580 with multidimensional statistical analyses (Fig. 2). ScRNAseq and snRNAseq are becoming
581 established as valuable tools to gain insight into basal⁹⁴ and reactive-astrocyte heterogeneity (Fig.
582 1e).^{40, 78, 95} Notably, isolation protocols may not always be optimal for astrocytes, resulting in low
583 numbers of cells or nuclei being sequenced, and some highly relevant but weakly-expressed
584 transcripts such as transcription factors and plasma-membrane receptors being overlooked,
585 particularly in snRNAseq. Translation from sc/snRNAseq data to *in situ* immunohistochemical
586 detection and functional validations is far from trivial, because the molecular profiles of astrocyte
587 clusters/subpopulations partly overlap. Thus, instead of individual markers, signatures composed
588 of a combination of markers with specified levels of expression or relative fold-changes are
589 required to identify astrocyte phenotypes.⁷⁴ Such signatures must be statistically validated to the
590 point of predicting phenotypes. Alternatively, the diversity within astrocyte populations from
591 mouse models may be dissected out by combining FACS and cell-surface markers identified in
592 screens.⁷¹ Further, emerging spatial transcriptomics that allow the simultaneous *in situ* detection of
593 numerous genes will be of value to study the heterogeneity of reactive astrocytes at local and
594 topographical levels (Fig. 1f).⁹⁶ Importantly, molecular signatures based on the expression of genes
595 or proteins need to be validated by assessing specific astrocyte functions (Table 2), since post-
596 transcriptional and post-translational events critically shape functional outcomes. Functional
597 validations should preferably be performed *in vivo*, or with *in vitro* models closely mimicking
598 human diseases. Classical knockout-, knockdown-, or CRISPR-based approaches to inactivate
599 gene expression are available to gain insight into the impact on disease of a given pathway within
600 previously identified astrocyte subsets.⁴⁰

601

602 *Signalling*

603

604 An important implication of the disease-specific induction of distinct reactive astrocyte states is
605 that the damage- and pathogen-associated stimuli from one disorder cannot be assumed to be active
606 in another. For example, the now widely-used cocktail of factors released by LPS-treated neonatal
607 microglia⁷² cannot be simply assumed to model reactive astrocytes in diseases other than neonatal
608 septic shock due to infection by gram-negative bacteria. Likewise, exposure to Tau, amyloid β or
609 α -synuclein needs to be carefully designed *in vivo* and *in vitro* to replicate the concentration, protein
610 species and combinations thereof found in patient brains. Acute metabolic damage with the
611 mitochondrial toxin MPTP does not replicate chronic PD, to cite another example of *in vivo*
612 inappropriate modelling. To complicate things further, the outcome of activating a signalling
613 pathway may depend on the upstream stimuli⁸² or priming caused by previous exposure to other
614 stimuli,⁹⁷ perhaps through epigenetic control.⁴⁰ Thus, careful selection of upstream stimuli is
615 essential for appropriate *in vivo* and *in vitro* modelling of disease-specific reactive astrocytes.
616 Finally, interventional strategies such as classical pharmacology,^{56, 98} genetic manipulation,^{42, 56}
617 and biomaterials⁹⁹ are available tools to modify pathological signalling in reactive astrocytes for
618 therapeutic purposes. Optogenetics²⁵ and Designer Receptor Exclusively Activated by Designer
619 Drugs (DREADD)²⁵ are potential tools to manipulate reactive astrocytes, or restore their aberrant
620 Ca^{2+} signalling observed in mouse models of neurodegenerative diseases.⁵³⁻⁵⁵ However, it is
621 unknown whether, and how, the changes in $\text{Na}^+/\text{K}^+/\text{Cl}^-/\text{Ca}^{2+}$ fluxes and second messengers
622 triggered by these approaches²⁵ modulate signalling cascades driving phenotypical changes of
623 reactive astrocytes (e.g., JAK-STAT and NF- κ B pathways).⁶

624

625 *Humanizing research*

626

627 Although some basic functional properties of astrocytes have been shown to be evolutionarily
628 conserved between humans and rodents,¹⁰⁰ it is still critical to study patient samples and develop
629 models of human reactive astrocytes because morphological and transcriptomic comparisons have

630 revealed prominent differences between mice and humans.¹⁰¹⁻¹⁰³ In addition to astrocytes from
631 *post-mortem* samples and biopsies (⁵⁹, Fig. 1b), hiPSC-derived astrocytes, which can be generated
632 with a fast protocol in 2D layers,¹⁰⁴ or integrated in 3D systems such as spheroids and organoids,<sup>105-
633 108</sup> are rapidly becoming commonplace in basic research^{11, 82} and therapy development.¹⁰⁹
634 Researchers need to be aware of the pros and cons of the various protocols available, as discussed
635 in previous sections and elsewhere.¹¹⁰⁻¹¹² Also, hiPSC glial mouse chimeric brains, in which hiPSC
636 differentiate into human astrocytes, oligodendrocytes, and their progenitors, offer the possibility to
637 study human astrocytes from patients in contexts amenable to *in vivo* experimentation.^{113, 114} In
638 addition, proteins released by injured astrocytes are currently being considered as fluid biomarkers
639 of neurotrauma.³¹ Biomarkers of reactive astrocytes in human disease will be indeed needed to
640 demonstrate target engagement of future astrocyte-directed therapies in clinical trials. Emerging
641 reactive-astrocyte biomarkers are either measured in blood or cerebrospinal fluid (e.g. YKL-40),¹¹⁵
642 or used for brain imaging such as MAO-B-based positron emission tomography (PET),¹¹⁶ which
643 provides important topographical information (Table 1).¹¹⁷ Plausibly, disease-specific biomarker
644 signatures rather than single ubiquitous biomarkers will be needed.

645

646 *Use of systems biology*

647

648 Computerised tools including systems biology and artificial intelligence are essential to organizing
649 and interpreting the increasing wealth of high-throughput multidimensional molecular and
650 functional data from reactive astrocytes. Currently, molecular data (e.g., -omics) can be
651 transformed into mathematical maps by artificial intelligence,¹¹⁸ thereby providing quantitative
652 representations of the otherwise vague notion of phenotypes. An example of functional data is 2D
653 and 3D Ca²⁺ imaging that generates kinetic profiles and maps for single astrocytes and 2D/3D
654 networks (Fig. 1c).^{119, 120} Artificial intelligence can identify patterns of Ca²⁺ signalling in
655 astrocytes.^{55, 120} Multidimensional molecular and functional data have then two applications. First,
656 multivariate analysis may unravel molecules, pathways and variables shaping astrocyte phenotypes
657 in acute versus chronic degenerative conditions, different disease stages, sexes, and CNS regions
658 (Fig. 2). Second, these data can be used to predict the net functional outcome of a complex mix of
659 potentially protective or deleterious pathways, and identification of hubs such as master
660 transcription factors or epigenetic regulators that, when activated, promote *globally* beneficial
661 transformations. Importantly, the inhibition of detrimental pathways must not secondarily impair
662 protective ones, or damage basic astrocyte functions. Finally, no astrocyte-targeting therapy can be
663 successful if it does not consider the complex interactions of reactive astrocytes with other CNS
664 cells.

665

666

667 **9. Concluding remarks**

668

669 The dawn of neuropathology in the late 19th and early 20th centuries witnessed widespread interest
670 in neuroglia. Today, research on astrocytes and their remodelling in the context of injury, disease,
671 and infection is undergoing a renaissance, with new researchers bringing exciting new techniques,
672 approaches, and hypotheses. Given the scarcity of disease-modifying treatments for chronic
673 diseases and acute injuries of the CNS, this astrocyte revival represents an opportunity to develop
674 largely unexplored therapeutic niches such as the manipulation of reactive astrocytes. However,
675 despite the substantial body of knowledge accumulated since the discovery of reactive astrocytes
676 a century ago, there are no therapies purposely designed against astrocyte-specific targets in clinical
677 practice. The present working consensus for research guidelines will hopefully boost more
678 coordinated and better focused efforts to improve, and therapeutically exploit, our knowledge about
679 the role(s) of reactive astrocytes in CNS diseases and injuries.

680

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702

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706 References

- 707
- 708 1. Virchow, R. *Cellular Pathology* (Robert M De Witt, New York, 1860).
- 709 2. Achucarro, N. Some pathological findings in the neuroglia and in the ganglion cells of the cortex
710 in senile conditions. *Bull Gov Hosp Insane* **2**, 81-90 (1910).
- 711 3. Andriezen, W.L. The neuroglia elements of the brain. *Brit. Med. J.* **2**, 227 - 230 (1893).
- 712 **The first account of hypertrophic reactive astrocytes in pathology, although they were not called**
713 **hypertrophic or reactive astrocytes.**
- 714 4. Weigert, C. Beiträge zur Kenntnis der normalen menschlichen Neuroglia. in *Zeitschrift für*
715 *Psychologie und Physiologie der Sinnesorgane* (ed. Liepmann) (Frankfurt 1895).
- 716 5. Del Río-Hortega, P. & Penfield, W.G. Cerebral cicatrix: The reaction of neuroglia and microglia
717 to brain wounds. *Bull Johns Hopkins Hosp* **41**, 278-303 (1927).
- 718 6. Escartin, C., Guillemaud, O. & Carrillo-de Sauvage, M.A. Questions and (some) answers on
719 reactive astrocytes. *Glia* **67**, 2221-2247 (2019).
- 720 7. Sofroniew, M.V. Astrocyte barriers to neurotoxic inflammation. *Nat Rev Neurosci* **16**, 249-263
721 (2015).
- 722 8. Verkhatsky, A., Zorec, R. & Parpura, V. Stratification of astrocytes in healthy and diseased brain.
723 *Brain Pathol* **27**, 629-644 (2017).
- 724 9. Messing, A., Brenner, M., Feany, M.B., Nedergaard, M. & Goldman, J.E. Alexander disease. *J*
725 *Neurosci* **32**, 5017-5023 (2012).
- 726 10. Brusilow, S.W., Koehler, R.C., Traystman, R.J. & Cooper, A.J. Astrocyte glutamine synthetase:
727 importance in hyperammonemic syndromes and potential target for therapy. *Neurotherapeutics* **7**, 452-470
728 (2010).
- 729 11. Lin, Y.T., *et al.* APOE4 causes widespread molecular and cellular alterations associated with
730 Alzheimer's disease phenotypes in human iPSC-derived brain cell types. *Neuron* **98**, 1141-1154 e1147
731 (2018).
- 732 **Technically improved generation of hiPSC-derived astrocytes demonstrates that astrocytes harboring a**
733 **genetic risk factor for AD are diseased astrocytes that may further exacerbate ongoing pathology.**
- 734 12. Eng, L.F., Vanderhaeghen, J.J., Bignami, A. & Gerstl, B. An acidic protein isolated from fibrous
735 astrocytes. *Brain Res* **28**, 351-354 (1971).
- 736 **The first identification of human GFAP in astrocytes from old multiple sclerosis plaques, post-leukotomy**
737 **scars, and the occipital and frontal horns of the lateral ventricles in old individuals with hydrocephalus ex**
738 **vacuo.**
- 739 13. Griemsmann, S., *et al.* Characterization of panglial gap junction networks in the thalamus,
740 neocortex, and hippocampus reveals a unique population of glial cells. *Cereb Cortex* **25**, 3420-3433
741 (2015).
- 742 14. Ben Haim, L. & Rowitch, D.H. Functional diversity of astrocytes in neural circuit regulation. *Nat*
743 *Rev Neurosci* **18**, 31-41 (2017).
- 744 15. Kriegstein, A. & Alvarez-Buylla, A. The glial nature of embryonic and adult neural stem cells.
745 *Annual review of neuroscience* **32**, 149-184 (2009).
- 746 16. Cahoy, J.D., *et al.* A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new
747 resource for understanding brain development and function. *J Neurosci* **28**, 264-278 (2008).
- 748 **This study represented a technical and conceptual breakthrough in the Neurosciences as the first unbiased**
749 **classification of brain cell populations based on transcriptomic profiles using early microarray analyses. The**
750 **resulting transcriptomes are a powerful tool to gain insight into novel brain cell functions. More recently,**
751 **the classification of brain cells has been further refined and enriched by sc/snRNAseq and spatial**
752 **transcriptomics.**
- 753 17. Roybon, L., *et al.* Human stem cell-derived spinal cord astrocytes with defined mature or reactive
754 phenotypes. *Cell reports* **4**, 1035-1048 (2013).
- 755 18. Rossi, D., *et al.* Focal degeneration of astrocytes in amyotrophic lateral sclerosis. *Cell death and*
756 *differentiation* **15**, 1691-1700 (2008).
- 757 19. Rodriguez, J.J., Terzieva, S., Olabarria, M., Lanza, R.G. & Verkhatsky, A. Enriched environment
758 and physical activity reverse astroglial degeneration in the hippocampus of AD transgenic mice. *Cell death*
759 *& disease* **4**, e678 (2013).

- 760 20. O'Callaghan, J.P., Brinton, R.E. & McEwen, B.S. Glucocorticoids regulate the synthesis of glial
761 fibrillary acidic protein in intact and adrenalectomized rats but do not affect its expression following brain
762 injury. *J Neurochem* **57**, 860-869 (1991).
- 763 21. Gerics, B., Szalay, F. & Hajos, F. Glial fibrillary acidic protein immunoreactivity in the rat
764 suprachiasmatic nucleus: circadian changes and their seasonal dependence. *J Anat* **209**, 231-237 (2006).
765 **Early demonstration that GFAP is regulated in a physiological context.**
- 766 22. Serrano-Pozo, A., Gomez-Isla, T., Growdon, J.H., Frosch, M.P. & Hyman, B.T. A phenotypic
767 change but not proliferation underlies glial responses in Alzheimer disease. *Am J Pathol* **182**, 2332-2344
768 (2013).
- 769 23. Wilhelmsson, U., *et al.* Redefining the concept of reactive astrocytes as cells that remain within
770 their unique domains upon reaction to injury. *Proc Natl Acad Sci U S A* **103**, 17513-17518 (2006).
771 **The complete visualization of astrocytes using whole-cell filling techniques revealed that reactive astrocytes**
772 **display subtle morphological changes and remain in their 3D territorial domain, highlighting that GFAP**
773 **immunostaining overestimates the true degree of astrocyte hypertrophy.**
- 774 24. Sosunov, A.A., *et al.* Phenotypic heterogeneity and plasticity of isocortical and hippocampal
775 astrocytes in the human brain. *J Neurosci* **34**, 2285-2298 (2014).
- 776 25. Yu, X., Nagai, J. & Khakh, B.S. Improved tools to study astrocytes. *Nat Rev Neurosci* **21**, 121-
777 138 (2020).
- 778 26. Schiweck, J., Eickholt, B.J. & Murk, K. Important shapeshifter: mechanisms allowing astrocytes
779 to respond to the changing nervous system during development, injury and disease. *Frontiers in cellular*
780 *neuroscience* **12**, 261 (2018).
- 781 27. Olabarria, M., Noristani, H.N., Verkhratsky, A. & Rodriguez, J.J. Concomitant astroglial atrophy
782 and astrogliosis in a triple transgenic animal model of Alzheimer's disease. *Glia* **58**, 831-838 (2010).
- 783 28. Black, J.A., Newcombe, J. & Waxman, S.G. Astrocytes within multiple sclerosis lesions
784 upregulate sodium channel Nav1.5. *Brain* **133**, 835-846 (2010).
- 785 29. Tachibana, M., *et al.* Clasmotodendrosis is associated with dendritic spines and does not represent
786 autophagic astrocyte death in influenza-associated encephalopathy. *Brain & development* **41**, 85-95
787 (2019).
- 788 30. Levine, J., *et al.* Traumatically injured astrocytes release a proteomic signature modulated by
789 STAT3-dependent cell survival. *Glia* **64**, 668-694 (2016).
- 790 31. Halford, J., *et al.* New astroglial injury-defined biomarkers for neurotrauma assessment. *J Cereb*
791 *Blood Flow Metab* **37**, 3278-3299 (2017).
792 **These data led to the first clinically used kit based on astrocyte-derived fluid biomarkers for neurotrauma**
793 **assessments.**
- 794 32. Ramon y Cajal, S. Contribución al conocimiento de la neuroglía del cerebro humano. *Trabajos del*
795 *Laboratorio de Investigaciones Biológicas de la Universidad de Madrid* **11**, 255 – 315 (1913).
- 796 33. Colombo, E., *et al.* Stimulation of the neurotrophin receptor TrkB on astrocytes drives nitric oxide
797 production and neurodegeneration. *The Journal of experimental medicine* **209**, 521-535 (2012).
798 **Demonstration that astrocytes may become neurotoxic by releasing nitric oxide.**
- 799 34. Theis, M., *et al.* Accelerated hippocampal spreading depression and enhanced locomotory activity
800 in mice with astrocyte-directed inactivation of connexin43. *J Neurosci* **23**, 766-776 (2003).
- 801 35. Kraft, A.W., *et al.* Attenuating astrocyte activation accelerates plaque pathogenesis in APP/PS1
802 mice. *FASEB J* **27**, 187-198 (2013).
- 803 36. Mucke, L., *et al.* Astroglial expression of human alpha(1)-antichymotrypsin enhances alzheimer-
804 like pathology in amyloid protein precursor transgenic mice. *Am J Pathol* **157**, 2003-2010 (2000).
805 **Early demonstration in a mouse model of AD that targeted manipulation of astrocyte functions by transgenic**
806 **tools has an impact on disease. A wealth of studies using transgenic mice and viral vectors followed suit,**
807 **and unequivocally demonstrate that reactive astrocytes influence CNS pathologies.**
- 808 37. Xu, L., Emery, J.F., Ouyang, Y.B., Voloboueva, L.A. & Giffard, R.G. Astrocyte targeted
809 overexpression of Hsp72 or SOD2 reduces neuronal vulnerability to forebrain ischemia. *Glia* **58**, 1042-
810 1049 (2010).
- 811 38. Furman, J.L., *et al.* Targeting astrocytes ameliorates neurologic changes in a mouse model of
812 Alzheimer's disease. *J Neurosci* **32**, 16129-16140 (2012).
- 813 39. Pardo, L., *et al.* Targeted activation of CREB in reactive astrocytes is neuroprotective in focal
814 acute cortical injury. *Glia* **64**, 853-874 (2016).

815 40. Wheeler, M.A., *et al.* MAFG-driven astrocytes promote CNS inflammation. *Nature* **578**, 593-599
816 (2020).
817 **The first study combining scRNAseq to characterize reactive astrocytes with targeted molecular**
818 **manipulations demonstrates, in a mouse model of MS, that reactive astrocytes are molecularly and**
819 **functionally heterogeneous, depending on brain area and disease stage.**

820 41. Bush, T.G., *et al.* Leukocyte infiltration, neuronal degeneration, and neurite outgrowth after
821 ablation of scar-forming, reactive astrocytes in adult transgenic mice. *Neuron* **23**, 297-308 (1999).
822 **The first demonstration that ablation of proliferative reactive astrocytes after stab wound injury in the mouse**
823 **forebrain is deleterious. This study made the case that astrocyte reactivity is not always detrimental as widely**
824 **believed, but may, instead, serve important homeostatic functions.**

825 42. Ceyzeriat, K., *et al.* Modulation of astrocyte reactivity improves functional deficits in mouse
826 models of Alzheimer's disease. *Acta neuropathologica communications* **6**, 104 (2018).

827 43. Reichenbach, N., *et al.* Inhibition of Stat3-mediated astrogliosis ameliorates pathology in an
828 Alzheimer's disease model. *EMBO molecular medicine* **11** (2019).

829 44. Kamphuis, W., *et al.* GFAP and vimentin deficiency alters gene expression in astrocytes and
830 microglia in wild-type mice and changes the transcriptional response of reactive glia in mouse model for
831 Alzheimer's disease. *Glia* **63**, 1036-1056 (2015).

832 45. Wheeler, M.A. & Quintana, F.J. Regulation of astrocyte functions in multiple sclerosis. *Cold*
833 *Spring Harbor perspectives in medicine* **9** (2019).

834 46. Colombo, E. & Farina, C. Astrocytes: key regulators of neuroinflammation. *Trends Immunol* **37**,
835 608-620 (2016).

836 47. Nobuta, H., *et al.* STAT3-Mediated astrogliosis protects myelin development in neonatal brain
837 injury. *Ann Neurol* (2012).

838 48. Anderson, M.A., *et al.* Astrocyte scar formation aids central nervous system axon regeneration.
839 *Nature* **532**, 195-200 (2016).

840 49. Herrmann, J.E., *et al.* STAT3 is a critical regulator of astrogliosis and scar formation after spinal
841 cord injury. *J Neurosci* **28**, 7231-7243 (2008).

842 50. Tyzack, G.E., *et al.* Astrocyte response to motor neuron injury promotes structural synaptic
843 plasticity via STAT3-regulated TSP-1 expression. *Nature communications* **5**, 4294 (2014).

844 51. Santello, M., Toni, N. & Volterra, A. Astrocyte function from information processing to cognition
845 and cognitive impairment. *Nat Neurosci* **22**, 154-166 (2019).

846 52. Semyanov, A., Henneberger, C. & Agarwal, A. Making sense of astrocytic calcium signals - from
847 acquisition to interpretation. *Nat Rev Neurosci* **21**, 551-564 (2020).

848 53. Jiang, R., Diaz-Castro, B., Looger, L.L. & Khakh, B.S. Dysfunctional calcium and glutamate
849 signaling in striatal astrocytes from Huntington's disease model mice. *J Neurosci* **36**, 3453-3470 (2016).

850 54. Kuchibhotla, K.V., Lattarulo, C.R., Hyman, B.T. & Bacskaï, B.J. Synchronous hyperactivity and
851 intercellular calcium waves in astrocytes in Alzheimer mice. *Science* **323**, 1211-1215 (2009).

852 55. Agarwal, A., *et al.* Transient opening of the mitochondrial permeability transition pore induces
853 microdomain calcium transients in astrocyte processes. *Neuron* **93**, 587-605 e587 (2017).
854 **Technically refined application of Ca²⁺ imaging approaches and machine learning unraveled dysregulation**
855 **of Ca²⁺ responses in a mouse model of ALS.**

856 56. Reichenbach, N., *et al.* P2Y1 receptor blockade normalizes network dysfunction and cognition in
857 an Alzheimer's disease model. *The Journal of experimental medicine* **215**, 1649-1663 (2018).

858 57. Habbas, S., *et al.* Neuroinflammatory TNFalpha impairs memory via astrocyte signaling. *Cell*
859 **163**, 1730-1741 (2015).
860 **This study illustrates how modulation of astrocyte signaling via TNFalpha can switch from physiological to**
861 **pathological.**

862 58. Tong, X., *et al.* Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in
863 Huntington's disease model mice. *Nat Neurosci* **17**, 694-703 (2014).
864 **Demonstration with targeted molecular manipulations that loss of astrocyte homeostatic functions**
865 **contributes to HD pathogenesis.**

866 59. Bedner, P., *et al.* Astrocyte uncoupling as a cause of human temporal lobe epilepsy. *Brain* **138**,
867 1208-1222 (2015).

868 60. Gomez-Arboledas, A., *et al.* Phagocytic clearance of presynaptic dystrophies by reactive
869 astrocytes in Alzheimer's disease. *Glia* **66**, 637-653 (2018).

- 870 61. Le Douce, J., *et al.* Impairment of glycolysis-derived L-serine production in astrocytes contributes
871 to cognitive deficits in Alzheimer's disease. *Cell metabolism* **31**, 503-517 e508 (2020).
- 872 62. Zhang, M., *et al.* Lactate deficit in an Alzheimer disease mouse model: the relationship with
873 neuronal damage. *Journal of neuropathology and experimental neurology* **77**, 1163-1176 (2018).
- 874 63. Jo, S., *et al.* GABA from reactive astrocytes impairs memory in mouse models of Alzheimer's
875 disease. *Nat Med* **20**, 886-896 (2014).
- 876 **Demonstration of astrocyte-targeted pharmacological manipulations to restore neural circuit homeostasis**
877 **by correcting production of GABA by astrocytes in an AD mouse model.**
- 878 64. Heo, J.Y., *et al.* Aberrant tonic inhibition of dopaminergic neuronal activity causes motor
879 symptoms in animal models of Parkinson's disease. *Curr Biol* **30**, 276-291 e279 (2020).
- 880 65. Chai, H., *et al.* Neural circuit-specialized astrocytes: transcriptomic, proteomic, morphological,
881 and functional evidence. *Neuron* **95**, 531-549 e539 (2017).
- 882 66. Zamanian, J.L., *et al.* Genomic analysis of reactive astrogliosis. *J Neurosci* **32**, 6391-6410 (2012).
883 **First evidence for molecular heterogeneity of reactive astrocytes using microarray-based transcriptomics of**
884 **acutely isolated astrocytes from mouse models of ischemia and septic shock. Studies in virtually all models**
885 **of CNS diseases followed.**
- 886 67. Orre, M., *et al.* Isolation of glia from Alzheimer's mice reveals inflammation and dysfunction.
887 *Neurobiol Aging* **35**, 2746-2760 (2014).
- 888 68. Sirko, S., *et al.* Astrocyte reactivity after brain injury-: The role of galectins 1 and 3. *Glia* **63**,
889 2340-2361 (2015).
- 890 69. Diaz-Castro, B., Gangwani, M.R., Yu, X., Coppola, G. & Khakh, B.S. Astrocyte molecular
891 signatures in Huntington's disease. *Science translational medicine* **11** (2019).
- 892 70. Itoh, N., *et al.* Cell-specific and region-specific transcriptomics in the multiple sclerosis model:
893 Focus on astrocytes. *Proc Natl Acad Sci U S A* **115**, E302-E309 (2018).
- 894 71. John Lin, C.C., *et al.* Identification of diverse astrocyte populations and their malignant analogs.
895 *Nat Neurosci* **20**, 396-405 (2017).
- 896 72. Liddelow, S.A., *et al.* Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*
897 **541**, 481-487 (2017).
- 898 73. Ransohoff, R.M. A polarizing question: do M1 and M2 microglia exist? *Nat Neurosci* **19**, 987-991
899 (2016).
- 900 74. Al-Dalahmah, O., *et al.* Single-nucleus RNA-seq identifies Huntington disease astrocyte states.
901 *Acta neuropathologica communications* **8**, 19 (2020).
- 902 75. Grubman, A., *et al.* A single-cell atlas of entorhinal cortex from individuals with Alzheimer's
903 disease reveals cell-type-specific gene expression regulation. *Nat Neurosci* **22**, 2087-2097 (2019).
- 904 76. Zhou, Y., *et al.* Human and mouse single-nucleus transcriptomics reveal TREM2-dependent and
905 TREM2-independent cellular responses in Alzheimer's disease. *Nat Med* **26**, 131-142 (2020).
- 906 77. Das, S., Li, Z., Noori, A., Hyman, B.T. & Serrano-Pozo, A. Meta-analysis of mouse
907 transcriptomic studies supports a context-dependent astrocyte reaction in acute CNS injury versus
908 neurodegeneration. *J Neuroinflammation* **17**, 227 (2020).
- 909 78. Habib, N., *et al.* Disease-associated astrocytes in Alzheimer's disease and aging. *Nat Neurosci*
910 (2020).
- 911 79. Henrik Heiland, D., *et al.* Tumor-associated reactive astrocytes aid the evolution of
912 immunosuppressive environment in glioblastoma. *Nature communications* **10**, 2541 (2019).
- 913 80. Varcianna, A., *et al.* Micro-RNAs secreted through astrocyte-derived extracellular vesicles cause
914 neuronal network degeneration in C9orf72 ALS. *EBioMedicine* **40**, 626-635 (2019).
- 915 81. di Domenico, A., *et al.* Patient-specific iPSC-derived astrocytes contribute to non-cell-
916 autonomous neurodegeneration in Parkinson's disease. *Stem cell reports* **12**, 213-229 (2019).
- 917 82. Tyzack, G.E., *et al.* A neuroprotective astrocyte state is induced by neuronal signal EphB1 but
918 fails in ALS models. *Nature communications* **8**, 1164 (2017).
- 919 83. Ledur, P.F., *et al.* Zika virus infection leads to mitochondrial failure, oxidative stress and DNA
920 damage in human iPSC-derived astrocytes. *Scientific reports* **10**, 1218 (2020).
- 921 84. Perriot, S., *et al.* Human induced pluripotent stem cell-derived astrocytes are differentially
922 activated by multiple sclerosis-associated cytokines. *Stem cell reports* **11**, 1199-1210 (2018).
- 923 85. Rodríguez-Arellano, J.J., Parpura, V., Zorec, R. & Verkhratsky, A. Astrocytes in physiological
924 aging and Alzheimer's disease. *Neuroscience* **323**, 170-182 (2016).

- 925 86. Jyothi, H.J., *et al.* Aging causes morphological alterations in astrocytes and microglia in human
926 substantia nigra pars compacta. *Neurobiol Aging* **36**, 3321-3333 (2015).
- 927 87. Rodriguez, J.J., *et al.* Complex and region-specific changes in astroglial markers in the aging
928 brain. *Neurobiol Aging* **35**, 15-23 (2014).
- 929 88. Cerbai, F., *et al.* The neuron-astrocyte-microglia triad in normal brain ageing and in a model of
930 neuroinflammation in the rat hippocampus. *PLoS One* **7**, e45250 (2012).
- 931 89. O'Callaghan, J.P. & Miller, D.B. The concentration of glial fibrillary acidic protein increases with
932 age in the mouse and rat brain. *Neurobiol Aging* **12**, 171-174 (1991).
- 933 90. Boisvert, M.M., Erikson, G.A., Shokhirev, M.N. & Allen, N.J. The aging astrocyte transcriptome
934 from multiple regions of the mouse brain. *Cell reports* **22**, 269-285 (2018).
- 935 91. Clarke, L.E., *et al.* Normal aging induces A1-like astrocyte reactivity. *Proc Natl Acad Sci U S A*
936 **115**, E1896-E1905 (2018).
- 937 92. Peters, O., *et al.* Astrocyte function is modified by Alzheimer's disease-like pathology in aged
938 mice. *J Alzheimers Dis* **18**, 177-189 (2009).
- 939 93. Childs, B.G., *et al.* Senescent cells: an emerging target for diseases of ageing. *Nature Reviews*
940 *Drug Discovery* **16**, 718-735 (2017).
- 941 94. Batiuk, M.Y., *et al.* Identification of region-specific astrocyte subtypes at single cell resolution.
942 *Nature communications* **11**, 1220 (2020).
- 943 95. Mathys, H., *et al.* Single-cell transcriptomic analysis of Alzheimer's disease. *Nature* **570**, 332-337
944 (2019).
- 945 **First snRNAseq analysis in human AD samples identifies sub-populations of reactive astrocytes.**
- 946 96. Chen, W.T., *et al.* Spatial transcriptomics and in situ sequencing to study Alzheimer's Disease.
947 *Cell* **182**, 976-991 (2020).
- 948 97. Hennessy, E., Griffin, E.W. & Cunningham, C. Astrocytes are primed by chronic
949 neurodegeneration to produce exaggerated chemokine and cell infiltration responses to acute stimulation
950 with the cytokines IL-1beta and TNF-alpha. *J Neurosci* **35**, 8411-8422 (2015).
- 951 98. Park, J.H., *et al.* Newly developed reversible MAO-B inhibitor circumvents the shortcomings of
952 irreversible inhibitors in Alzheimer's disease. *Science advances* **5**, eaav0316 (2019).
- 953 99. Zuidema, J.M., Gilbert, R.J. & Gottipati, M.K. Biomaterial approaches to modulate reactive
954 astroglial response. *Cells Tissues Organs* **205**, 372-395 (2018).
- 955 100. Bedner, P., Jabs, R. & Steinhauser, C. Properties of human astrocytes and NG2 glia. *Glia* **68**, 756-
956 767 (2020).
- 957 101. Zhang, Y., *et al.* Purification and characterization of progenitor and mature human astrocytes
958 reveals transcriptional and functional differences with mouse. *Neuron* **89**, 37-53 (2016).
- 959 **First study reporting transcriptomes of human astrocytes, paving the way for the highly used open-source**
960 **database of gene expression for all brain cell types in humans and mice (<https://www.brainrnaseq.org/>)**
- 961 102. Oberheim, N.A., *et al.* Uniquely hominid features of adult human astrocytes. *J Neurosci* **29**, 3276-
962 3287 (2009).
- 963 103. Oberheim, N.A., Wang, X., Goldman, S. & Nedergaard, M. Astrocytic complexity distinguishes
964 the human brain. *Trends Neurosci* **29**, 547-553 (2006).
- 965 104. Tchieu, J., *et al.* NFIA is a gliogenic switch enabling rapid derivation of functional human
966 astrocytes from pluripotent stem cells. *Nature biotechnology* **37**, 267-275 (2019).
- 967 105. Sloan, S.A., *et al.* Human astrocyte maturation captured in 3D cerebral cortical spheroids derived
968 from pluripotent stem cells. *Neuron* **95**, 779-790 e776 (2017).
- 969 106. Lancaster, M.A., *et al.* Cerebral organoids model human brain development and microcephaly.
970 *Nature* **501**, 373-379 (2013).
- 971 107. Quadrato, G., *et al.* Cell diversity and network dynamics in photosensitive human brain organoids.
972 *Nature* **545**, 48-53 (2017).
- 973 108. Giandomenico, S.L., *et al.* Cerebral organoids at the air-liquid interface generate diverse nerve
974 tracts with functional output. *Nat Neurosci* **22**, 669-679 (2019).
- 975 109. Colombo, E., *et al.* Siponimod (BAF312) activates Nrf2 while hampering NFkappaB in human
976 astrocytes, and protects from astrocyte-induced neurodegeneration. *Frontiers in immunology* **11**, 635
977 (2020).
- 978 110. Hirbec, H., *et al.* Emerging technologies to study glial cells. *Glia* **68**, 1692-1728 (2020).
- 979 111. Guttenplan, K.A. & Liddelow, S.A. Astrocytes and microglia: Models and tools. *The Journal of*
980 *experimental medicine* **216**, 71-83 (2019).

981 112. Almad, A. & Maragakis, N.J. A stocked toolbox for understanding the role of astrocytes in
982 disease. *Nature reviews. Neurology* **14**, 351-362 (2018).

983 113. Han, X., *et al.* Forebrain engraftment by human glial progenitor cells enhances synaptic plasticity
984 and learning in adult mice. *Cell stem cell* **12**, 342-353 (2013).

985 114. Osipovitch, M., *et al.* Human ESC-derived chimeric mouse models of Huntington's disease reveal
986 cell-Intrinsic defects in glial progenitor cell differentiation. *Cell stem cell* **24**, 107-122 e107 (2019).

987 115. Craig-Schapiro, R., *et al.* YKL-40: a novel prognostic fluid biomarker for preclinical Alzheimer's
988 disease. *Biol Psychiatry* **68**, 903-912 (2010).

989 116. Carter, S.F., *et al.* Evidence for astrocytosis in prodromal Alzheimer disease provided by 11C-
990 deuterium-L-deprenyl: a multitracer PET paradigm combining 11C-Pittsburgh compound B and 18F-
991 FDG. *J Nucl Med* **53**, 37-46 (2012).

992 **Non invasive imaging of reactive astrocytes in human patients.**

993 117. Carter, S.F., *et al.* Astrocyte biomarkers in Alzheimer's disease. *Trends Mol Med* **25**, 77_95
994 (2019).

995 118. Romeo-Guitart, D., *et al.* Neuroprotective drug for nerve trauma revealed using artificial
996 intelligence. *Scientific reports* **8**, 1879 (2018).

997 119. Bindocci, E., *et al.* Three-dimensional Ca²⁺ imaging advances understanding of astrocyte
998 biology. *Science* **356** (2017).

999 120. Wang, Y., *et al.* Accurate quantification of astrocyte and neurotransmitter fluorescence dynamics
1000 for single-cell and population-level physiology. *Nat Neurosci* **22**, 1936-1944 (2019).

1001 121. Ben Haim, L., *et al.* The JAK/STAT3 pathway is a common inducer of astrocyte reactivity in
1002 Alzheimer's and Huntington's diseases. *J Neurosci* **35**, 2817-2829 (2015).

1003 122. Hol, E.M. & Pekny, M. Glial fibrillary acidic protein (GFAP) and the astrocyte intermediate
1004 filament system in diseases of the central nervous system. *Current opinion in cell biology* **32**, 121-130
1005 (2015).

1006 123. Moreels, M., Vandenabeele, F., Dumont, D., Robben, J. & Lambrechts, I. Alpha-smooth muscle
1007 actin (alpha-SMA) and nestin expression in reactive astrocytes in multiple sclerosis lesions: potential
1008 regulatory role of transforming growth factor-beta 1 (TGF-beta1). *Neuropathology and applied
1009 neurobiology* **34**, 532-546 (2008).

1010 124. Jing, R., *et al.* Synemin is expressed in reactive astrocytes in neurotrauma and interacts
1011 differentially with vimentin and GFAP intermediate filament networks. *J Cell Sci* **120**, 1267-1277 (2007).

1012 125. Yamada, T., Kawamata, T., Walker, D.G. & McGeer, P.L. Vimentin immunoreactivity in normal
1013 and pathological human brain tissue. *Acta Neuropathol* **84**, 157-162 (1992).

1014 126. Gui, Y., Marks, J.D., Das, S., Hyman, B.T. & Serrano-Pozo, A. Characterization of the 18 kDa
1015 translocator protein (TSPO) expression in post-mortem normal and Alzheimer's disease brains. *Brain
1016 Pathol* **30**, 151-164 (2020).

1017 127. Wilhelmus, M.M., *et al.* Specific association of small heat shock proteins with the pathological
1018 hallmarks of Alzheimer's disease brains. *Neuropathology and applied neurobiology* **32**, 119-130 (2006).

1019 128. Furman, J.L., *et al.* Blockade of astrocytic calcineurin/NFAT signaling helps to normalize
1020 hippocampal synaptic function and plasticity in a rat model of traumatic brain injury. *J Neurosci* **36**, 1502-
1021 1515 (2016).

1022 129. Michetti, F., *et al.* The S100B story: from biomarker to active factor in neural injury. *J
1023 Neurochem* **148**, 168-187 (2019).

1024 130. Sun, W., *et al.* SOX9 is an astrocyte-specific nuclear marker in the adult brain outside the
1025 neurogenic regions. *J Neurosci* **37**, 4493-4507 (2017).

1026 131. Wanner, I.B., *et al.* Glial scar borders are formed by newly proliferated, elongated astrocytes that
1027 interact to corral inflammatory and fibrotic cells via STAT3-dependent mechanisms after spinal cord
1028 injury. *J Neurosci* **33**, 12870-12886 (2013).

1029 132. Campbell, S.C., *et al.* Potassium and glutamate transport is impaired in scar-forming tumor-
1030 associated astrocytes. *Neurochem Int* **133**, 104628 (2020).

1031 133. Voss, C.M., *et al.* AMP-activated protein kinase (AMPK) regulates astrocyte oxidative
1032 metabolism by balancing TCA cycle dynamics. *Glia* **68**, 1824-1839 (2020).

1033 134. Kimbrough, I.F., Robel, S., Roberson, E.D. & Sontheimer, H. Vascular amyloidosis impairs the
1034 gliovascular unit in a mouse model of Alzheimer's disease. *Brain* **138**, 3716-3733 (2015).

1035 135. Deshpande, T., *et al.* Subcellular reorganization and altered phosphorylation of the astrocytic gap
1036 junction protein connexin43 in human and experimental temporal lobe epilepsy. *Glia* **65**, 1809-1820
1037 (2017).

1038 136. Frakes, A.E., *et al.* Microglia induce motor neuron death via the classical NF-kappaB pathway in
1039 amyotrophic lateral sclerosis. *Neuron* **81**, 1009-1023 (2014).

1040 137. Eraso-Pichot, A., *et al.* GSEA of mouse and human mitochondriomes reveals fatty acid oxidation
1041 in astrocytes. *Glia* **66**, 1724-1735 (2018).

1042 138. Machler, P., *et al.* In vivo evidence for a lactate gradient from astrocytes to neurons. *Cell*
1043 *metabolism* **23**, 94-102 (2016).

1044 139. Lerchundi, R., Huang, N. & Rose, C.R. Quantitative imaging of changes in astrocytic and
1045 neuronal adenosine triphosphate using two different variants of ATeam. *Frontiers in cellular neuroscience*
1046 **14**, 80 (2020).

1047 140. Ioannou, M.S., *et al.* Neuron-astrocyte metabolic coupling protects against activity-induced fatty
1048 acid toxicity. *Cell* **177**, 1522-1535 e1514 (2019).

1049 141. Polyzos, A.A., *et al.* Metabolic reprogramming in astrocytes distinguishes region-specific
1050 neuronal susceptibility in Huntington mice. *Cell metabolism* **29**, 1258-1273 e1211 (2019).

1051 142. Oe, Y., Akther, S. & Hirase, H. Regional distribution of glycogen in the mouse brain visualized
1052 by immunohistochemistry. *Adv Neurobiol* **23**, 147-168 (2019).

1053 143. Vezzoli, E., *et al.* Ultrastructural evidence for a role of astrocytes and glycogen-derived lactate in
1054 learning-dependent synaptic stabilization. *Cereb Cortex* **30**, 2114-2127 (2020).

1055 144. Vicente-Gutierrez, C., *et al.* Astrocytic mitochondrial ROS modulate brain metabolism and mouse
1056 behaviour. *Nature Metabolism* **1**, 201–211 (2019).

1057 145. Damisah, E.C., *et al.* Astrocytes and microglia play orchestrated roles and respect phagocytic
1058 territories during neuronal corpse removal in vivo. *Science advances* **6**, eaba3239 (2020).

1059 146. Simonovitch, S., *et al.* Impaired autophagy in APOE4 astrocytes. *J Alzheimers Dis* **51**, 915-927
1060 (2016).

1061 147. Goetzl, E.J., *et al.* Traumatic brain injury increases plasma astrocyte-derived exosome levels of
1062 neurotoxic complement proteins. *FASEB J* **34**, 3359-3366 (2020).

1063 148. Orre, M., *et al.* Reactive glia show increased immunoproteasome activity in Alzheimer's disease.
1064 *Brain* **136**, 1415-1431 (2013).

1065 149. Sirko, S., *et al.* Reactive glia in the injured brain acquire stem cell properties in response to sonic
1066 hedgehog glia. *Cell stem cell* **12**, 426-439 (2013).

1067 150. Buffo, A., *et al.* Origin and progeny of reactive gliosis: A source of multipotent cells in the injured
1068 brain. *Proc Natl Acad Sci U S A* **105**, 3581-3586 (2008).

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1071 **Figure legends**

1072 **Figure 1. Multivariate assessment of reactive astrocytes**

1073 **a.** Reactive astrocyte proliferation in the vicinity of blood vessels assessed by co-staining for BrdU
1074 (green, arrows), DAPI (blue), GFAP (white), and CD31 (red) after stab injury of the mouse cortex.
1075 Bar size: 15 μm . Unpublished image from Drs. Sirko and Götz.

1076 **b.** Human cortical protoplasmic astrocytes in a surgical specimen injected with Lucifer yellow
1077 (arrow, injection site) that traverses the gap junctions into neighbouring astrocytes. Bar size: 45
1078 μm . Courtesy of Drs. Xu, Sosunov, and McKhann, Columbia University Department of
1079 Neurosurgery.

1080 **c.** Event-based determination of Ca^{2+} responses in a GCaMP6-expressing astrocyte (surrounded by
1081 a dashed line) in mouse cortical slices using Astrocyte QUantitative Analysis (AQuA).¹²⁰ Colours
1082 indicate AQuA events occurring in a single 1-sec frame of a 5-min movie. Bar size: 10 μm .

1083 **d.** Activation of the transcription factor STAT3 (green) assessed by nuclear accumulation in
1084 GFAP⁺ reactive astrocytes (red) surrounding an amyloid plaque (blue, arrow) in a mouse AD
1085 model. Bar size: 20 μm . Adapted from ¹²¹.

1086 **e.** ScRNAseq in the remission phase of a mouse MS model reveals several transcriptional astrocyte
1087 clusters. These astrocyte sub-populations may be validated with spatial transcriptomics, as shown
1088 in f in an AD model. Adapted from ⁴⁰.

1089 **f.** Distribution of 87 astrocytic (green), neuronal (red), microglial (yellow), and oligodendroglial
1090 (blue) genes as shown with *in situ* multiplex gene sequencing in a coronal section from a mouse
1091 AD model. The method ‘reads’ barcodes of antisense DNA probes that simultaneously target
1092 numerous mRNAs. Bar size: 800 μm . Boxed area is magnified in bottom image, showing 6E10⁺
1093 amyloid- β plaques (white, arrows). Adapted from ⁹⁶.

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1096 **Fig. 2. Workflow for the identification of key variables shaping astrocyte reactivity using**
1097 **multidimensional analyses**

1098 **a.** Variables to *measure* in individual experiments. Although at present it is unrealistic to measure
1099 all in the same experiment, it will in most cases be possible to measure at least two or three.

1100 **b.** Variables to *record* in individual experiments. In some experiments, all or most of these
1101 variables are kept constant and are not compared, but they should all be recorded to allow for future
1102 comparison across experiments and studies.

1103 **c.** Individual studies will generate multidimensional datasets of reactive astrocytes that can be
1104 organized in matrices containing all outcome measures of variables assessed in (a) (e.g. omics data,
1105 functional measurements). One matrix may be generated for each condition listed in (b) using data
1106 obtained in a. Determining whether such states are equivalent to fixed categories rather than
1107 temporary changes due to the dynamic nature of cell functioning requires cross-comparison among
1108 studies or longitudinal studies, paired with statistical analyses (d).

1109 **d.** Multidimensional data analysis and clustering statistics of weighted scores from datasets (a)
1110 across different contexts (b) represented in matrices (c) allow identification of functional vectors
1111 (V) driving astrocyte reactivity in different contexts. A high score and a low score in each vector
1112 represent gain and loss of function, respectively. The graph shows a hypothetical plot of simulated
1113 multivariate datasets from (a) (each dot represents one dataset/sample) obtained in different
1114 contexts (b), depicted in different colours. Astrocytes with shared features segregate together along
1115 three axes according to the predominance of the function represented in each vector. A state is
1116 defined by where the dataset(s) falls in the V1-3 space. The analysis can be n-dimensional, but for
1117 visual clarity, we show a 3-dimensional scenario.

Table 1. Potential markers of reactive astrocytes

Marker	Known function	Type of change	Conditions observed	Species	Comments	Ref
Cytoskeleton						
GFAP	Intermediate filament	↑ mRNA & protein	Widespread. Not in some trauma models	Widespread	Released by injured astrocytes Cleavage product found in CSF/plasma (neurotrauma biomarker)	122
Nestin	Intermediate filament	↑ mRNA & protein	AD, AxD, MS, spinal cord injury, TBI	Hu, Ms	Also a marker of progenitor cells	123
Synemin	Intermediate filament	↑ mRNA & protein	AD, AxD, astrocytoma, TBI	Hu, Ms	Normally expressed in a subset of astrocytes during development	124
Vimentin	Intermediate filament	↑ mRNA & protein	Widespread	Widespread	Also expressed by endothelial cells, vascular smooth muscle cells, and immature astrocytes	125
Metabolism						
ALDOC	Glycolytic enzyme	↑ protein	SCI, TBI	Hu, Ms	Released by injured astrocytes Fluid biomarker for neurotrauma	30, 31
BLBP/ FABP7	Lipid transport	↑ protein	AD, MS, TBI	Hu, Ms	Also a marker of immature astrocytes. Released by injured astrocytes. Fluid biomarker for neurotrauma	31, 60
MAO-B	Catecholamine catabolic enzyme	↑ protein	AD, ALS, PD	Hu, Ms	PET radiotracers available Also expressed by catecholaminergic neurons	63, 64, 117
TSP0	Mitochondrial lipid transporter	↑ mRNA & protein	AD, MS, ischemia	Hu, Rt, Ms	PET radiotracers available. Also induced in reactive microglia. Expressed by vascular cells	126
Chaperones						
CRYAB	Chaperone activity	↑ mRNA & protein, ↑ secretion	AD, AxD, epilepsy, HD, MS, TBI	Hu, Ms	Reduces protein aggregation	74, 95
HSPB1/ HSP27	Chaperone	↑ mRNA & protein	AD, AxD, epilepsy, MS, tauopathies, stroke	Widespread		95, 127
Secreted proteins						

C3	Complement factor	↑ mRNA & protein	ND, prion disease, septic shock	Hu, Ms	Also expressed by microglia	72
CHI3L1/ YKL40	Unclear function	↑ mRNA & protein ↑ secretion	Widespread	Hu, Ms	Increase in CSF is a prognostic biomarker in LOAD and MS	79, 115
Lcn2	Iron trafficking protein	↑ mRNA & protein	AxD, MS, septic shock, ALS, stroke	Widespread		66
Serpina3n/ ACT	Serine protease inhibitor	↑ mRNA	AD, septic shock, stroke	Hu, Ms	Secreted to extracellular matrix	66
MT	Metal binding	↑ mRNA & protein	HD, PD, AD	Hu, Ms	Antioxidant effects	74
THBS-1	Synaptogenic factor	↑ mRNA & protein ↑ secretion	Axotomy, MS	Hu, Ms	STAT3-regulated. Has beneficial synaptogenic effects	50
Cell signalling – Transcription factors						
NFAT	Transcription factor	↑ mRNA, protein, nuclear translocation	AD, TBI, PD	Hu, Ms	Links Ca ²⁺ signalling with reactive transcriptional changes	38, 128
NTRK2/ TrkB IL17R	Receptors	↑ mRNA and/or protein	Epilepsy, MS (white matter)	Hu, Ms	Trigger non-canonical pathological BDNF-dependent signalling, and/or NF-κB activation and NO production	33, 109
S100B	Ca ²⁺ binding protein	↑ protein and release	Widespread	Widespread	Released upon injury. Fluid biomarker	129
SOX9	Transcription factor	↑ mRNA and/or protein	ALS, stroke, SCI	Hu, Ms	Nuclear staining Also present in ependymal cells and in neurogenic niches	130
STAT3	Transcription factor	Phosphorylation, nuclear translocation	Widespread	Widespread	Also expressed in neurons and other cell types	49, 50, 131
Channels - Transporters						
EAAT1 & 2	Glutamate transporters	↓ mRNA, protein and uptake	ND	Widespread	May be also detected in some neurons	53, 132
KIR4.1	K ⁺ channel	↓ mRNA & protein	Widespread	Hu, Ms	May or may not translate into alteration of K ⁺ buffering	58

Abbreviations used: AD: Alzheimer's disease; ALS: amyotrophic lateral sclerosis; AxD: Alexander disease; BDNF: Brain-derived neurotrophic factor; CSF: cerebrospinal fluid; HD: Huntington's disease; Hu: human; LOAD: late onset AD; MS: multiple sclerosis; Ms: Mouse; ND: neurodegenerative disease; NO: nitric oxide; PET: positron emission tomography; PD: Parkinson's disease; Rt: rat; SCI: spinal cord injury; TBI: traumatic brain injury.

This table lists potential markers for reactive astrocytes in different pathological contexts in human diseases and animal models. The list is not meant to be exhaustive; other markers exist and more will be added over time. These proteins can be used to further characterize the reactive state of astrocytes, although note that, like GFAP (see Section 3), none of these proteins should be used as a single or universal marker of reactive astrocytes, nor for the time being do they identify a specific type of reactive astrocyte. Plausibly, markers in the table will be part of signatures defining disease-specific or core markers of reactive astrocytes, as well as astrocyte-based fluid biomarkers (see Section 8). Importantly, few of these markers are astrocyte-specific; therefore, additional methods to identify or isolate astrocytes and remove contamination by other cell-types will be in order.

Table 2. Potential functional assessments for reactive astrocytes

Function/Phenomenon	Potential readouts	Ref
Ca²⁺ signalling in single cells Ca²⁺ based network dynamics	Ca ²⁺ imaging with chemical or genetically-encoded Ca ²⁺ indicators	25, 52, 55, 119, 120
Ionic homeostasis	Measurement of ionic currents and membrane potential (electrophysiology). Direct measurement of extracellular K ⁺ levels	58, 132
Glutamate, GABA, D-serine and ATP release Glutamate uptake and conversion	Detection of neuroactive factors using fluorescent sensors and <i>in vivo</i> two-photon imaging Quantification of neuroactive factors in extracellular milieu and CSF (FRET, HPLC, CE-LIF, fluorescent sensors like GluSnFR, enzymatic kits)	25
	Analysis of glutamate currents (electrophysiology) and/or transporter content (immunoblot, immunostainings)	109, 132
	Metabolism of ¹³ C-labeled substrates (GC-MS & HPLC)	133
Astrocyte inter-cellular connectivity	Diffusion of permeant dyes in astrocyte networks (patch-clamp & imaging), FRAP	59
Vascular coupling Maintenance of BBB integrity	Assessment of vascular responses after Ca ²⁺ uncaging or optogenetic stimulation of astrocytes (two-photon imaging, optical intrinsic imaging, MRI)	134
	Assessment of BBB permeability with detection in the parenchyma of blood proteins or dyes (Evans blue, Dextrans)	135
Signalling Transcription factor activation	Standard biochemical assays. Signalling manipulation by DREADDs Transcription factor translocation and DNA binding assays, chromatin immunoprecipitation, reporters	25, 109, 136
Production of synaptogenic and neurotrophic factors, ECM, cytokines, chemokines	Synapse quantification <i>in vivo</i> and upon exposure to astrocyte-conditioned media <i>in vitro</i> Proteomics/metabolomics of astrocyte-conditioned media and acutely sorted astrocytes Multiplex ELISA assays, immunostainings	72, 97
Interactions with neurons, oligodendrocytes, OPC and microglia	<i>In vivo/ex vivo</i> analyses, co-cultures or exposure to conditioned media and assessment of function/survival	58, 72, 82
Glycolysis Fatty-acid oxidation Lactate production Glycogen metabolism Mitochondrial respiration	Metabolism of ³ H/ ¹⁴ C/ ¹³ C/- labelled energy substrates (GC-MS, radioactive assays, NMR)	133, 137
	Glucose, pyruvate, lactate and ATP quantification with genetically-encoded fluorescent sensors and <i>in vivo</i> two-photon imaging	138, 139
	Lipid-droplet and fatty-acid staining with BODIPY dyes	140

	NADH imaging (FLIM)	141
	Activities of electron transport chain complexes Extracellular acidification, oxygen consumption (Sea Horse, voltametry)	141
	Quantification of glycogen granules by EM or immunostainings	142, 143
NO-ROS production/detoxification	NO/ROS imaging with intra/extracellular fluorescent sensors or probes Immunostaining for oxidized residues Activity of antioxidant enzymes with commercial kits	33, 144
Endolysosomal system	Detection of phagocytosed materials (array tomography, EM, 2 photon microscopy) Uptake of myelin debris or labelled synaptosomes	60, 72, 145
	Autophagic flux	81, 146
	Exosome production	80, 147
	Proteasome/lysosome proteolytic activity (fluorescent probes)	148
Proliferation	BrdU incorporation Ki67, PCNA, cyclin labelling (calculation of a proliferative index, i.e. % of positive cells in the population) Characterization of astrocyte progeny by fate mapping	149, 150
Scar-border formation	Morphometric/functional analyses (e.g. composition, permeability to immune cells)	131
Abbreviations used: BBB: blood-brain barrier; BrdU: bromodeoxyuridine; CE-LIF: capillary electrophoresis with laser induced fluorescent detection, CSF: cerebrospinal fluid; DREADD: designer receptor exclusively activated by designer drugs. ECM: Extracellular matrix; EM: electron microscopy; FLIM: fluorescence lifetime imaging microscopy; FRAP: Fluorescence recovery after photobleaching. FRET: Förster resonance energy transfer; GC-MS: gas chromatography-mass spectrometry; HPLC: high performance liquid chromatography; NO: nitric oxide; NMR: nuclear magnetic resonance; OPC: oligodendrocyte progenitor cells; PCNA: proliferating cell nuclear antigen; ROS: reactive oxygen species.		

The table depicts assays that can be performed in astrocytes to characterize their functional properties. References and functions are not exhaustive and aim to illustrate the existing methodology by providing recent protocols for each approach. Although most references concern studies in healthy or reactive astrocytes, some additional tools relevant to reactive astrocytes are listed as well. Assays can be performed in human neurosurgical samples, *in vivo*, or in acute brain slices of animal models and/or *in vitro* (pure cultures, mixed cultures, organoids). Note that some assays require specific equipment and skills or the physical isolation of astrocytes to measure astrocyte-specific functional parameters. No reference is provided for enzymatic assays that are commercially available.

BOX 1. Basic consensus and recommendations for research on reactive astrocytes

BASIC CONSENSUS

1. Reactive astrocytes are astrocytes that undergo morphological, molecular, and functional changes in response to pathological situations in surrounding tissue (CNS disease/injury/deleterious experimental manipulation).
2. Astrocytes with disease-causing genetic mutations are diseased astrocytes that initiate or contribute to pathology, and later become reactive in ways that may differ from the astrocyte reactivity normally triggered by external stimuli. Genetic polymorphisms linked to CNS diseases may also influence astrocytic functions and prime astrocytes to acquire distinct reactive states.
3. There is no prototypical reactive astrocyte, nor do reactive astrocytes polarize into simple binary phenotypes, such as good/bad, neurotoxic/neuroprotective, A1/A2, etc. Rather, reactive astrocytes may adopt multiple states depending on context, with only a fraction of common changes between different states.
4. Loss of some homeostatic functions, and gain of some protective or detrimental functions, may happen simultaneously. Whether the overall impact on disease is beneficial or detrimental will be determined by the balance and nature of lost and gained functions, and the relative abundance of different astrocyte subpopulations.

RECOMMENDATIONS

4. Astrocyte phenotypes should be defined by a combination of molecular markers (Table 1) and functional readouts (Table 2), preferably *in vivo*. GFAP and morphology alone are not sufficient criteria to qualify astrocytes as reactive.
5. The specifics of the astrocytes under study should be spelled out in titles, abstracts, and results of articles (e.g., X-positive astrocytes in Y region showed Z phenomenon).
6. Multivariate and clustering analysis of molecular and functional data will facilitate the identification of distinct phenotypes of reactive astrocytes (Fig. 2).
7. Local, regional, temporal, subject/patient, and sexual heterogeneity of reactive astrocytes should be studied (Fig. 2).
8. The discovery and validation of plasma/serum and cerebrospinal fluid biomarkers, as well as of PET radiotracers of astrocyte reactivity, is a research priority, as it will facilitate astrocyte-directed drug development.

Figure 1

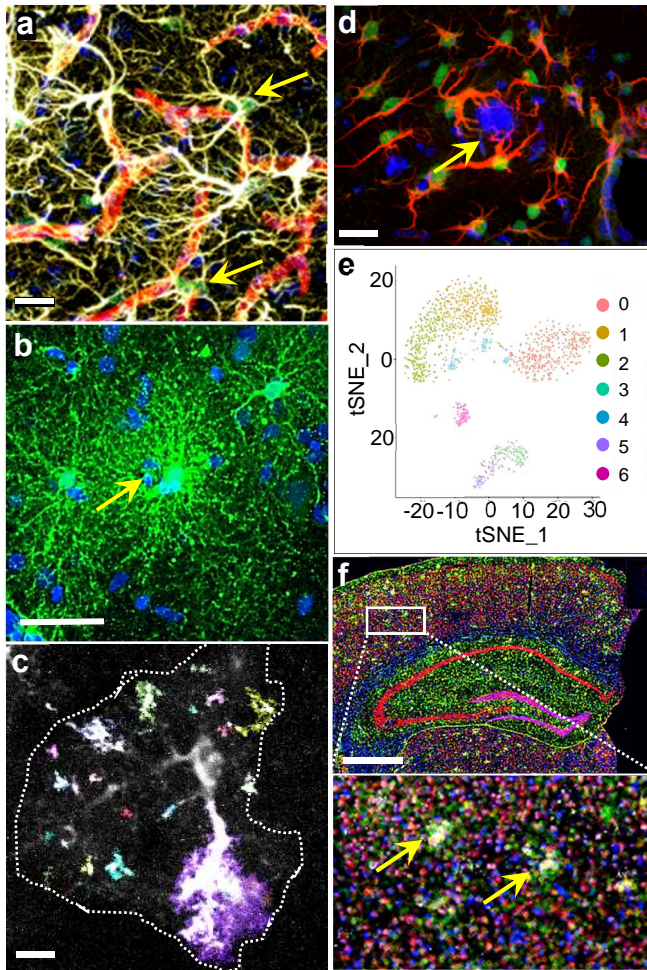


Figure 2

