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A ROADMAP TO INTEGRATE ASTROCYTES

INTO SYSTEMS NEUROSCIENCE

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Running title: Astrocytic Ca^{2+} signaling and neuronal coding

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Abstract

Systems Neuroscience is still mainly a neuronal field, despite the plethora of evidence supporting the fact that astrocytes modulate local neural circuits, networks, and complex behaviors. In this article, we sought to identify which types of studies are necessary to establish whether astrocytes, beyond their well-documented homeostatic and metabolic functions, perform computations implementing mathematical algorithms that sub-serve coding and higher-brain functions. First, we reviewed Systems-like studies that include astrocytes in order to identify computational operations that these cells may perform, using Ca^{2+} transients as their encoding language. The analysis suggests that astrocytes may carry out canonical computations in a time scale of sub-seconds to seconds in sensory processing, neuromodulation, brain state, memory formation, fear, and complex homeostatic reflexes. Next, we propose a list of actions to gain insight into the outstanding question of which variables are encoded by such computations. The application of statistical analyses based on machine learning, such as dimensionality reduction and decoding in the context of complex behaviors, combined with connectomics of astrocyte-neuronal circuits, are, in our view, fundamental undertakings. We also discuss technical and analytical approaches to study neuronal and astrocytic populations simultaneously, and the inclusion of astrocytes in advanced modeling of neural circuits, as well as in theories currently under exploration such as predictive coding and energy-efficient coding. Clarifying the relationship between astrocytic Ca^{2+} and brain coding may represent a leap forward towards novel approaches in the study of astrocytes in health and disease.

Key words: Astrocytes, energy-efficient coding, decoding, dimensionality reduction, predictive coding.

67   **Main points**

68   • Astrocytes may use Ca2+ signals to perform canonical computations in complex behaviors
69   on a time scale of sub-seconds to seconds.
70
71   • Statistical tools from Systems Neuroscience could be used to unravel variables and
72   algorithms encoded by astrocytic Ca^{2+}.

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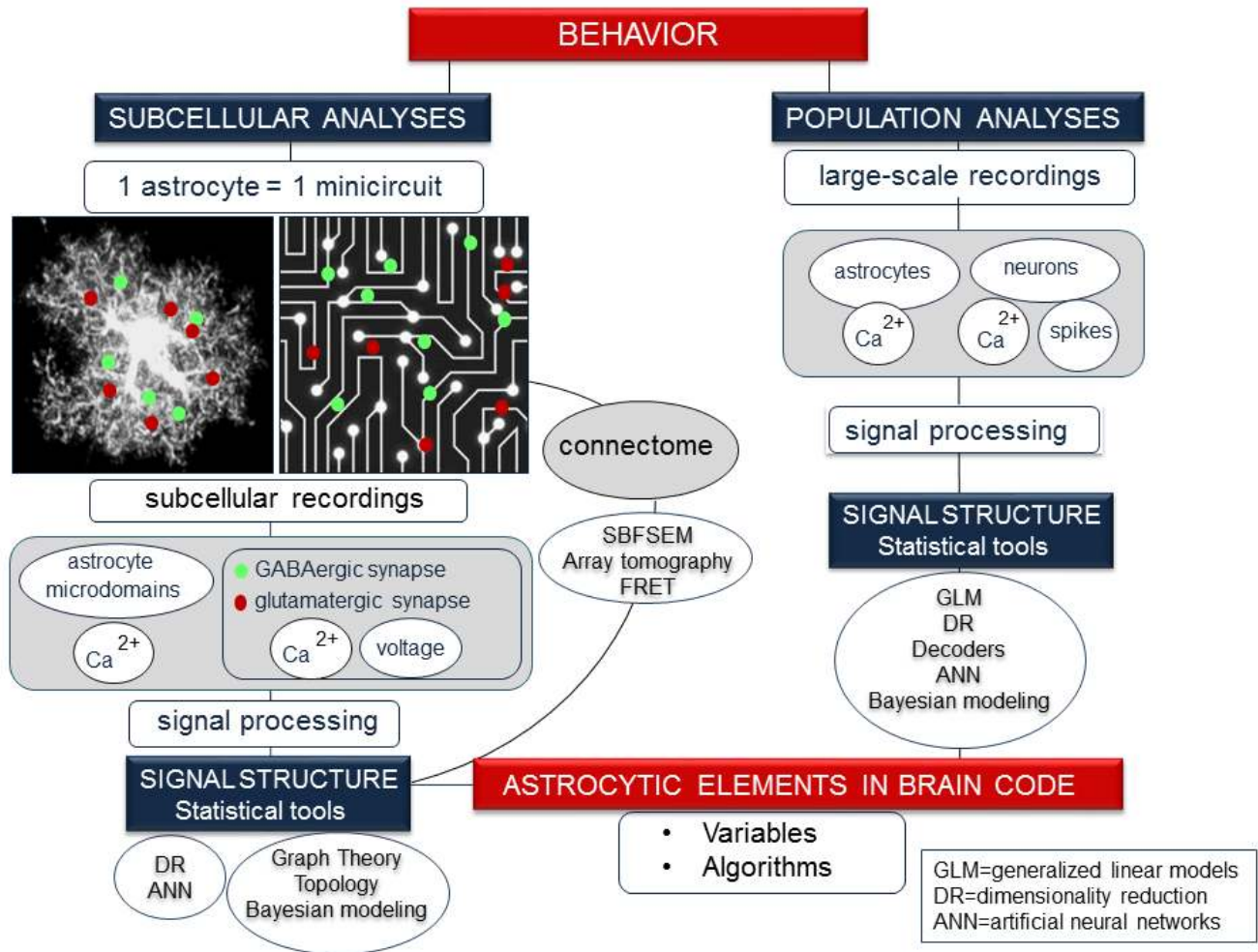
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1. Systems Neuroscience is primarily a neuronal field

The study of the central nervous system (CNS) encompasses different levels of analysis: molecular, cellular, anatomical, behavioral, cognitive and systems. Systems Neuroscience aims at integrating these former fields, which have mostly grown independently. For example, Molecular Neuroscience has traditionally focused on the smallest functional level without a connection to cognition, whereas Behavioral Psychology and Psychophysics have typically studied cognition separately from its molecular and neuronal underpinnings. The overarching goal of Systems Neuroscience is to understand how neural circuits give rise to cognitive functions, emotions and behavior by *simultaneously* recording neuronal activity and behavior at the highest spatiotemporal resolution possible.

Systems Neuroscience is arguably a field of neurons. A proof of this can be found in recent editions (2015-2017) of the three international conferences dedicated to Systems and Computational Neuroscience—here we will not dwell on what is 'Systems' and what 'Computational' since the two fields are highly overlapping and complementary. The conferences are the 'Conference and Workshop on Neural Information Processing Systems' (NIPS), the 'Organization for Computational Neurosciences' (OCNS) and 'Computational and Systems Neuroscience (COSYNE). Of approximately 3000 communications, fewer than 1% included non-neuronal cells. The pervasive use of the phrase 'neural circuit' in the programs most of the time refers to computational integration of information embedded in neuronal biophysical substrates. The scarce attention to non-neuronal cells is puzzling, at least from the perspective of the astrocyte field, given the evidence that astrocytes contribute to circuit-based phenomena at the synaptic (Araque et al., 2014) and network (Poskanzer & Yuste, 2016) levels. Although efforts are being made in the US Brain Initiative and the European Human Brain Project to develop studies incorporating non-neuronal cells, it seems as though progress in astrocyte biology has advanced in parallel to systems neuroscience, and astrocytes have been excluded from unified theories of brain function, as previously noted (Poskanzer & Molofsky, 2018). Although extensive modeling of astrocytic Ca^{2+} fluxes exists (Manninen et al., 2018), and sporadic studies have explored the application of astrocyte-based computations to artificial intelligence (Alvarellos-Gonzalez et al., 2012; Porto-Pazos et al., 2011), astrocytes are characteristically missing from advanced *in silico* modeling of neural circuits (Capone et al., 2017; Deneve et al., 2017; Gjorgjieva et al., 2016; Markram et al., 2015).

Is this exclusion justified because the mechanisms underlying the well documented impact of astrocytes on neural circuits fall within the realm of intercellular signaling, homeostasis and metabolism, which, although essential for the maintenance of neural circuits, may not qualify as 'computing' processes? Or, are astrocytes fundamental to the computational foundations of the brain? Later we will elaborate on what is and what is not computation, but rather than struggling to define 'computation' we ask, instead, whether processes that take place in astrocytes participate in the implementation by neural circuits of processes sub-serving coding, complex behaviors, and higher-brain functions. In other words, if computation is an emerging property of a given neural network (Yuste, 2015), do astrocytes help to shape such property beyond providing metabolic and homeostatic support? If they do, specific questions are whether there are niche(s) in Systems Neurosciences that would particularly profit from astrocyte idiosyncrasies, and whether the impressive techniques and theoretical armamentarium deployed by Systems Neuroscience could be used—and are sufficient—to unravel possible astrocyte-based computations. In an early article in Computational Neuroscience, it was argued that anatomical features provide valuable insights about how the CNS operates because '*the nervous system is a product of*

evolution, not design. The computational solutions evolved by nature may be unlike those that humans would invent, if only because evolutionary changes are always made within the context of a design and architecture that already is in place (Sejnowski et al., 1988). It follows that the unique anatomical arrangement between astrocytes and neurons might be part of computational solutions refined by evolution that have made the brain a highly efficient task-performing system. In this article we will explore the possible computations carried out by astrocytes. First, we will succinctly describe the fundamentals (section 2) and current challenges (sections 3 and 4) of Systems Neuroscience. We will continue by reviewing Systems-like studies involving astrocytes (sections 5 and 6). We will then propose a to-do list to further integrate astrocytes in Systems Neurosciences, thus helping to dissipate the historical and perhaps no longer tenable gap between astrocytes and neurons (section 7). We do not touch upon other glial cells because, as discussed earlier (Masgrau et al., 2017), the cells grouped under this name are molecularly and morphologically distinct; hence, their contribution to higher-brain functions deserves individual attention.

2. **Computational foundations of the CNS**

What is computation? When we say that the brain computes we mean that it creates and stores representations of physical and conceptual entities, and performs operations on these representations in order to carry out discrete tasks underlying behavior. The goal of Computational/Systems Neuroscience is to describe these processes in mathematical and computational terms. In this framework, it is believed that the mathematical treatment of the representations is possible precisely because computation implies abstraction, thus permitting generation of internal models of the world using biophysical substrates (Marr, 1976). The action of generating representations is known as *encoding* because the brain converts physical and conceptual entities into a code, that is, a combination of symbols representing variables. Symbols can be discrete, continuous and distributed among numerous neurons and brain areas. A prime example of what is and is not computation can be found in action potentials. Their generation is caused by fine homeostatic adjustments of membrane voltage that *per se* may not qualify as a computation (Stuart et al., 1997), but complex combinations of action potentials constitute the 'symbols' of the 'alphabet' used by the brain to compute. Examples of variables encoded by the brain are the position, color and shape features of a given object (Seymour et al., 2010), sound categories (Tsunada & Cohen, 2014), the distance between the eyes in face recognition (Chang & Tsao, 2017), and the reward value of a choice during decision making (Saez et al., 2018). The information embedded in neural biophysical substrates can be *decoded* and transferred ('rerouted'), possibly transformed into different formats and neural substrates. Examples are the on-line holding of memory during decision making (Hasson et al., 2015), and memory replay during memory consolidation (Foster, 2017). It is worth stressing that the current computational view of the brain is not an established truth, but a simplified framework highly influenced by information theory, computer science and linguistics to guide experimental testing.

Computation takes place at several hierarchically organized levels. Levels include from brain areas, nuclei, maps, columns, circuits, single neurons, and sub-neuronal compartments, such as dendrites, spines, somas and axons (Mesulam, 1998). Levels, moreover, interact in specific temporal and topological patterns (Betzel & Bassett, 2017) (Vidaurre et al., 2017). A hierarchical organization is, in essence, a modular organization of computation (D. Meunier et al., 2009), such that a successful general theory of the brain will have to explain how tasks performed at one module(s) give rise to tasks performed by the larger module(s). Currently, a widely assumed premise is that most components of cognition emerge from

171 the level of transiently active circuits—some authors prefer to speak about ensembles of neurons or cell
172 assemblies (Buzsaki, 2010)—whose dynamics arise, in turn, from complex interactions involving the
173 three classical building blocks: neuronal intrinsic excitability, synaptic efficiency, and connectivity
174 (Gjorgjieva et al., 2016). Simply put, circuit dynamics within the range of millisecond to minutes control
175 fast behaviors such as perception and decision making (Khani & Rainer, 2016), whereas synaptic changes
176 lasting hours and days control learning and memory (Sweatt, 2016). Connectivity includes two main
177 patterns: feed-forward, supporting a unidirectional flow of information, and recurrent, composed of
178 positive and negative feedbacks that lead to self-sustained multiple activity patterns (Duarte et al., 2017).
179 Connections are mostly selective but they can be random as well, giving rise to complex, slow dynamics
180 that include chaotic interactions (Mastrogiuseppe & Ostojic, 2018). Another widely assumed premise is
181 that local circuits, however dynamic, are too anatomically fixed to adapt their behavior to contexts that
182 need to be globally broadcast, for instance, sleep-wake cycles, mood, reward, and attention during
183 perception and decision making. To circumvent this problem, neuromodulation has been suggested as a
184 solution. Neuromodulation refers to the relatively rapid (in the range of seconds) functional
185 reconfiguration of circuits throughout the brain by acetylcholine, dopamine, noradrenaline and
186 serotonin, which are released by subcortical and brainstem nuclei: the *nucleus basalis* of Meynert (NBM),
187 the striatum, the *locus coeruleus,* and the Raphe nucleus (Avery & Krichmar, 2017). Neuromodulation
188 participates in working memory, attention, brain state and plasticity (C. N. Meunier et al., 2017; Sara,
189 2009; Thiele & Bellgrove, 2018).

190 *Neural substrates of brain computations*. The ultimate goal of Systems/Computational Neuroscience is
191 to explain how electrical and chemical signals are used in the brain to represent and process information
192 (Sejnowski et al., 1988). Currently, a widely accepted assumption is, as noted, that external variables are
193 encoded into action potentials. Theories and empirical evidence point to firing rates (average number of
194 action potentials per unit of time)(Gerstner et al., 1997), action-potential timing (length of time between
195 action potentials) (Panzeri et al., 2001), population coding (joint activity of several neurons) (Panzeri et
196 al., 2015), and neural dynamics (the way electrical activities evolve with time and space) (Shenoy et al.,
197 2013), as potential features of action potentials that, in infinite amount of combinations, have enough
198 breadth to constitute the basis of the brain code(s). A key implication of the multi-level organization of
199 the brain is that code(s) are multi-level, too. This means that external variables are encoded by the
200 collective activity of numerous simpler elements, which carry either synergistic or complementary
201 information (Panzeri et al., 2015). This principle is the driving premise in population and dynamic coding,
202 and has informed the development of methods for recording from large populations of neurons, including
203 multi-electrode arrays, which can record up to 10^3 neurons (Einevoll et al., 2012), Ca^{2+} imaging, which
204 can simultaneously record over 10^4 neurons (Sofroniew et al., 2016)(Pachitariu et al., 2016), and
205 functional resonance magnetic imaging (fRMI), which makes use of BOLD (blood-oxygen-level contrast
206 imaging) to unravel functional connectivity among regions encompassing over 10^5 neurons (Fox &
207 Raichle, 2007). It is worth stressing that the measurable signals in the latter two approaches are not
208 action potentials, but single-cell Ca^{2+} rises and regional oxygen consumption, respectively. Although the
209 premise for using large-scale Ca^{2+} imaging in neurons is that single-neuron Ca^{2+} signals represent slower
210 non-linear encoding of the underlying action potentials (Vogelstein et al., 2010) (Lutcke et al., 2013),
211 non-electrical signals, as well as global voltage oscillations measured with field potentials and
212 electroencephalograms, plausibly carry additional information that is computationally relevant. For
213 example, it has been proposed that synaptic facilitation mediated by neuronal Ca^{2+} signals sustains
214 working memory (Mongillo et al., 2008). All in all, biophysical substrates of brain computations other

215 than the ones directly or indirectly based on neuronal activity will plausibly arise in the future, including,
216 we posit, astrocyte-based computations.
217
218 *Contemporary brain theories*. According to the number of publications, one of the most influential brain
219 frameworks is predictive coding, which aim to account for core principles underlying adaptive circuit
220 remodeling. The key tenets of predictive coding are the following. *First*, representationalism, the brain
221 operates by building models of the outer world, conceptual categories and expected outcomes of actions.
222 *Second*, evaluation of new information against embedded models is at the core of many brain operations
223 besides decision making, including perceptual discrimination, voluntary selective attention and learning.
224 *Third*, the nature of such evaluations is probabilistic, since the underlying algorithms weigh in pros and
225 cons and similarity of the novel information with respect to internal models. A central notion is that
226 *'organisms care less about representing what is actually out there in the world than about how this*
227 *reality conflicts with their predictions about what should be there'* (Fitch, 2014). An apparent virtue of
228 this strategy is minimization of data storage since it takes fewer bits to represent the mean and deviations
229 from it than to attempt *de novo* representations (Fitch, 2014). *Fourth*, the brain tries to minimize its
230 prediction errors such that internally-generated predictions are constantly optimized with external inputs
231 in an iterative process. In predictive coding, neuromodulation is proposed as computing part of the
232 statistics of errors made by predictions (Lau et al., 2017; Stephan et al., 2015). The bulk of empirical
233 support for predictive coding lies in the domains of perception, reward learning, and decision making, as
234 documented in humans, monkeys, and rodents (Summerfield et al., 2008; Wacongne et al., 2011) (Kok &
235 de Lange, 2014; Markov et al., 2014) (Diederen et al., 2017; Nasser et al., 2017) (Leinweber et al., 2017),
236 whereas the framework appears to be under exploration in memory consolidation (Cross et al., 2018) and
237 emotion (Barrett, 2017). Other general CNS frameworks worth mentioning are global workspace theory,
238 which describes the basic circuit from which consciousness emerges (Baars, 2005), and liquid computing,
239 which states that neural circuits have the capacity to store information of previous perturbation(s),
240 analogous to the ripples generated on the surface of a pond when stones are thrown into it (Maass et al.,
241 2002). Finally, influential theoretical constructions about basic operative principles of the brain—
242 compatible with global frameworks—include brain oscillations (Buzsaki & Draguhn, 2004), efficient
243 coding (Chalk et al., 2018), energy-efficient coding (Laughlin, 2001), neural integrators (Mazurek et al.,
244 2003), inhibitory/excitatory balance (Brunel, 2000; Litwin-Kumar & Doiron, 2012), noise (Arieli et al.,
245 1996), and circuit degeneracy (Sporns, 2013).
246
247 **3. Challenges, obstacles, and growth areas in Systems Neuroscience.**
248
249 Despite the progress in the last decade, understanding brain computations remains a central challenge
250 of modern Neuroscience. The readily observable behavioral variables that are used experimentally to
251 study brain encoding, for instance, rewards, choices and stimulus features, represent the tip of the
252 iceberg, because the vast majority of variables used by the brain in complex behaviors and higher-brain
253 functions, are typically latent. However, this should not distract us from the impressive predictive power
254 that analytical tools are achieving in Systems Neuroscience. Examples of success can be found in
255 neuroprosthetics, where the electrical activity of the brain of a human user is decoded into motor
256 commands (Cangelosi & Invitto, 2017); decision-making, in which decision outputs can be predicted from
257 action potentials with 80% accuracy in monkeys before a response is observed (Kiani et al., 2014), and
258 with 70% accuracy in rats, even before stimulus onset (Nogueira et al., 2017), and face recognition. Here,
259 the face seen by a Rhesus monkey can be reproduced with 90% accuracy by tracking neuronal activity in

260 the inferior temporal cortex (Chang & Tsao, 2017). Although the achievements are remarkable, there is
261 still room to improve these numbers. In the workflow of Systems Neuroscience from signal capture to
262 deciphering the brain code, areas of improvement include signal recording, signal processing, data
263 analyses, and astrocyte-focused studies (Fig. 1). Key issues are briefly described next.

265 *Data load in large-scale recordings.* The trend of improving predictions by simultaneously recording
266 more neurons has created a serious challenge: the ever-increasing size of the data seriously hampers
267 storage, processing and analysis. In order to simplify and reduce data size of recordings, several methods
268 exist to extract low-dimensional mathematical representations from multi-neuronal electrical recordings
269 (Aljadeff et al., 2016; Cunningham & Yu, 2014). The obstacle is all the more complex in Ca^{2+} imaging,
270 which has become a dominant method for recording from large populations of neurons, because special
271 methods are necessary to extract the coarse-grained and noisy Ca^{2+} data prior to data analysis. Algorithms
272 such as Suite2p (Pachitariu, 2016), and CNMF (Constrained and/or nonnegative matrix factorization,
273 (Pnevmatikakis et al., 2016)), represent advances in the simplification of imaging data processing prior
274 analysis. Caveats of current calcium imaging data processing are discussed in (Stringer & Pachitariu,
275 2018). Alternatively, shot-gun statistics unravels network connectivity information from recording at
276 only 10% of the neurons at a given time, thus simplifying the experimental load of large-scale recordings
277 (Soudry et al., 2015). Data-sharing and collaborative solutions have been proposed as well to manage the
278 surge of data (Paninski & Cunningham, 2018).

280 *Statistical tools for understanding data.* The standard problem is to determine how behavioral variables
281 are encoded by neurons, and how this information is decoded, either by downstream neurons, or by an
282 external observer. Different statistical tools address encoding and decoding. For encoding, generalized
283 linear models (GLMs), a generalization of multiple linear regression, regress neuronal activity against
284 behavioral variables to determine the set of variables that explain more neuronal activity (Aljadeff et al.,
285 2016) (Nogueira et al., 2017). Decoding techniques, typically linear classifiers (Arandia-Romero et al.,
286 2017; Quian Quiroga & Panzeri, 2009), as well as more recent artificial neural networks (ANNs) (Paninski
287 & Cunningham, 2018) are used to predict, trial-by-trial, values of behavioral variables from neuronal
288 activity, either using single neuronal activity, or the individual activity of large neuronal populations
289 recorded from multi-electrode-arrays or Ca^{2+} imaging. These methods are supervised machine learning
290 tools because both behavioral and neuronal variables are preselected and labelled. Also, unsupervised
291 tools such as dimensionality reduction have been developed, and used in parallel, in order to reduce data
292 complexity by identifying low-dimensional latent factors, where relevant behavioral variables could be
293 represented (Cunningham & Ghahramani, 2015). Of note, detection of relevant subspaces of neuronal
294 activity, and optimal selection of behavioral features to regress against neuronal data, will facilitate the
295 discovery of computational principles. An elegant example is the aforementioned study by (Chang & Tsao,
296 2017), in which successful face identification in non-human primates was possible with 50-dimensional
297 data, and recordings of 200 neurons. Likewise, feature selection can be adaptively improved with
298 artificial intelligence (Yamins & DiCarlo, 2016). As with signal processing, data load is a challenge in
299 signal analysis, for the number of observations per condition does not necessarily grow in parallel with
300 the growth of complexity and number of dimensions of the data. For example, recording 20 neurons for
301 30 min produces the same number of observations *per* neuron than recording 1000 neurons during the
302 same amount of time, but the number of dimensions increases 50-fold with the larger neuronal
303 population. This means that encoding, decoding and dimensionality reduction techniques need to be

304 constrained by specific structural and anatomical knowledge of the neural substrates to be operationally
305 useful.
306
307 *Optogenetics and chemogenetics.* These anatomically precise and reversible tools allow establishing
308 cause-effect relationships between the electrical activity of single neurons, or neuronal populations, and
309 behavioral parameters. Optogenetics is based on the expression of light-sensitive regulators of
310 transmembrane conductance (ion channels and chloride pumps) coupled with fiber optic- and laser
311 diode-based light delivery (Boyden et al., 2005; Li et al., 2005). Cell type specificity is accomplished by
312 targeting the light sensitive channels with cell-type specific promoters. Light-activation of neurons
313 expressing channels like channelrhodopsins (ChR1, ChR2) result in neuronal depolarizations due to
314 import of cations such as Na^+, K^+, and Ca^{2+}—the latter at trace levels. By contrast, optical stimulations of
315 archaerhodopsin (Arch) and halorhodopsins (NpHR) pumps cause hyperpolarization of neurons by
316 exporting H^+, or by importing chloride ions, respectively. An alternative approach to classic opsins is the
317 light-sensitive G-coupled receptor, also called OptoGq/Gs, which modulates receptor-initiated
318 biochemical signaling pathways (Airan et al., 2009). Chemogenetics is based on the use of Designer
319 Receptors Exclusively Activated by Designer drugs (DREADDs), a family of G protein-coupled receptors
320 (GPCRs) that are solely activated by a pharmacologically inert drug, clozapine N-oxide (CNO) (Alexander
321 et al., 2009). DREADDs can also be targeted to neurons with viral or transgenic delivery systems using
322 neuron-specific promoters. Relevant insights into behavior, cognition and basic brain homeostasis have
323 been gained with neuron-targeted optogenetic and chemogenetic approaches (Deisseroth, 2015) (Roth,
324 2016).

325 *Subcellular computations.* Increasing the number of recorded neurons may not be the only solution for
326 obtaining better data. Insofar each and every neuron must integrate and convert thousands of synaptic
327 inputs into a single output (London & Hausser, 2005), concerns have been raised about the
328 oversimplification of neurons as 'integrate-and-fire' nodes in large-scale recordings and *in silico*
329 simulations, and a plea exists to pay renewed attention to the great computational potency of single
330 neurons (Fitch, 2014). Spine computations and biophysical substrates are reviewed in (Yuste, 2013), and
331 a recent example of the computational relevance of dendritic shafts is the finding that non-linear
332 dynamics based on dendritic conductance can help sharpen time and rate codes in grid cells, thereby
333 improving the accuracy of space representation (Schmidt-Hieber et al., 2017). In the context of imaging,
334 voltage dyes represent a growth area allowing for recording at subcellular resolution at multiple points
335 along dendrites and axons (Xu et al., 2017). The data, combined with whole-cell reconstructions with
336 electron microscopy (Vishwanathan et al., 2017), will arguably improve the understanding of dendritic
337 computations and network connectivity.
338
339 *A need for theoretical frameworks and modeling.* The wealth of descriptive data will not advance
340 knowledge unless analyses are guided by hypotheses and complemented with modeling.
341 Computational/Systems Neuroscience is thus engaged in a virtuous cycle whereby data generate models,
342 and models make predictions that can be tested *ad infinitum* against new proposed experiments. The
343 trade-offs of increasing the realism of models by incorporating more biophysical variables *versus*
344 developing simplifying models, as discussed in (Sejnowski et al., 1988), are still debated (Marder, 2015).
345 Whatever the approach, *in vivo* models, and their *in silico* counterparts, need to be informed by large-
346 scale hypotheses combined with simpler questions, in order to advance on the outstanding question of
347 how the brain processes information with such energetic efficiency. We discussed the remarkable

348 production of studies informed by predictive coding and other theoretical constructions. Other theories
349 will plausibly arise in the future.
350
351 **4.   Astrocyte-based computations as a growth area in Systems Neuroscience.**
352
353 We posit that variables used in brain coding may be partially embedded in astrocyte biophysical
354 substrates, such that the incorporation of astrocytes as computational building blocks in neural circuits
355 may help advance Systems Neurosciences. Significant gaps of knowledge, however, exist. *First*, there is
356 no evidence that astrocytes gate, transform, store and reroute information in the brain by carrying out
357 processes that can be described in abstract mathematical terms. Astrocytes do participate in brain state
358 (Poskanzer & Yuste, 2016), neuromodulation (Magistretti & Morrison, 1988) (Paukert et al., 2014)
359 (Srinivasan et al., 2015), and in a wide variety of naturally-occurring recurrent circuits, where they have
360 been proposed as carrying out spatiotemporal integration of multicellular inputs (Araque et al., 2014).
361 Examples indeed exist of discrimination and integration of synaptic information by astrocytes (Perea &
362 Araque, 2005), but the underlying algorithms and their behavioral correlates remain undetermined.
363 *Second*, if astrocytes compute, are Ca^{2+} transients a biophysical substrate of astrocyte-based
364 computations? The intuition that they are already exists in the field, resting on a wealth of studies that,
365 since the 1990s, have used Ca^{2+} imaging to assess astrocyte activation at increasing spatiotemporal
366 resolution, thanks to the unremitting refinement of fluorescent indicators and optical imaging (reviewed
367 in Kastanenka et al.(K. V. Kastanenka, Arbel-Ornath, M., Hudry, E., Galea, E., Xie, H., Backskai, B.J.,
368 2016) and (Bazargani & Attwell, 2016)). However, although *in silico* modeling documents that astrocytes
369 can encode extracellular cues into variables in Ca^{2+} transients (De Pitta et al., 2008), the statistical
370 methods currently used to encode and decode neuronal action potentials (Section 3) have not been
371 applied to astrocyte data obtained *in vivo*. *Third*, it is not known whether the subcellular Ca^{2+}
372 microdomains in astrocytes would carry out different functions within distinct circuits associated with
373 different complex behaviors, nor whether astrocytes would perform similar computations throughout the
374 brain, or are as functionally heterogeneous as neurons. It is worth mentioning that in the last decade
375 controversies have arisen concerning the regulation and consequences of Ca^{2+} signaling in astrocytes.
376 Specifically, whether Ca^{2+} comes from endoplasmic reticulum and mitochondria, or from the extracellular
377 milieu, the very notion of Ca^{2+}-dependent gliotransmission, the role of astrocytes in long-term
378 potentiation (LTP), and whether D-serine is a gliotransmitter have been debated—reviewed in (Bazargani
379 & Attwell, 2016; Savtchouk & Volterra, 2018). Currently, the prevailing notion reconciling these
380 discrepancies is that Ca^{2+} responses are highly complex and context-dependent, such that the signaling
381 leading to Ca^{2+} rises, the sub-cellular source of such Ca^{2+}, the speed of transients, as well as the
382 downstream effects, are dependent on the subcellular astrocyte compartment(s), and the neural circuit
383 (Savtchouk & Volterra, 2018). In this piece we do not focus on mechanistic issues, but rather on whether
384 and how astrocytes may perform computations using Ca^{2+} transients.

385 **5.   Systems-like studies in astrocytes**

386 A prototypical study in Systems Neuroscience includes three components: (i) recording of electrical
387 activity in multiple neurons, (ii) computerized analysis to decode information embedded in action-
388 potential firings, and (iii) simultaneous measurement of a cognitive or behavioral function. The statistical
389 analyses reveal correlations and, increasingly often, causal relationships between changes in patterns of
390 neuronal-population firing and specific behavioral or cognitive responses (Sections 2 and 3). There are

391 no studies, to our knowledge, recording the Ca²⁺ activity of multiple astrocytes, followed by analysis with
392 GLM or decoders in the context of a behavioral paradigm defined by distinct features that can be
393 correlated with patterns of astrocytic Ca²⁺ activity. Among studies linking astrocytes and behavior (for
394 reviews (Oliveira et al., 2015; Santello et al., 2019)), in section 5.1 we discuss the ones closer to the neuron-
395 focused experimental design in Systems Neuroscience, for they include recordings of Ca²⁺-based
396 astrocyte excitability, as well as electrical or optical recordings of neuronal activity, in the context of
397 complex behaviors or neuromodulation. Conversely, in Section 5.2 we focus on studies showing
398 modulation of local brain circuits associated with complex behaviors, or brain state, by *transient*
399 optogenetic or chemogenetic astrocyte activation. In section 6, we extract computational lessons from
400 these studies, and identify gaps of knowledge, taking into account, when appropriate, previous and recent
401 studies that, although lacking any of the aforementioned components, support our computational
402 insights. Table 1 summarizes the analysis. In Fig.1 we highlight in red approaches within the general
403 workflow of Systems Neuroscience including signal capture, processing and analysis that could be used
404 with astrocytic data.

405 *5.1. Activation of Ca²⁺ transients in astrocytes by sensory stimulation and neuromodulation*

406 Studies in the mouse barrel cortex have shown activation of Ca²⁺ in astrocyte somata after whisker
407 stimulation using fluorescent Ca²⁺ dyes (X. Wang et al., 2006) (Takata et al., 2011) and genetically-
408 encoded Ca²⁺ indicators (Stobart et al., 2018). Astrocytic Ca²⁺ increases are delayed with respect to Ca²⁺
409 rises in neurons (Stobart et al., 2018). Also, astrocytic Ca²⁺ rises are dependent on whisker stimulation
410 frequency, and they are blocked by inhibitors of metabotropic glutamate receptors, indicating that they
411 are caused by glutamate released from neurons (X. Wang et al., 2006). Whisker stimulation-dependent
412 Ca²⁺ rises in astrocytes are detected as early as at 2 s when dyes are used, and at 120 ms in the case of
413 faster, genetically encoded indicators, although peak responses range between 3-12 s regardless of the
414 Ca²⁺ indicator. Likewise, visual stimulation triggers neuron-dependent somatic Ca²⁺ transients in
415 astrocytes in the visual cortex of ferret, with a delay of 1-3 s and a peak at 6 s (Schummers et al., 2008).
416 Importantly, the latter study demonstrates that astrocyte activation is highly tuned to orientation maps
417 at a single-cell resolution, and documents that astrocytes mediate hemodynamic signals in the visual
418 cortex, which was confirmed in another study in the barrel cortex (Stobart et al., 2018). The study by
419 (Takata et al., 2011) is also relevant because it demonstrates the following. First, cholinergic
420 neuromodulation originating in the NBM potentiates the activation of local field potentials elicited by
421 whisker stimulation. Second, neuromodulation is strictly dependent on Ca²⁺ rises in astrocytes, as shown
422 by the disappearance of neuronal-activity potentiation in mice lacking IP3R2-dependent signaling.
423 Crucially, abrogation of Ca²⁺ signaling in astrocytes in these mice shifts brain state to a desynchronized
424 mode, as assessed with local field potentials in cortex. The impact of cholinergic neuromodulation on
425 astrocyte Ca²⁺ responses is also documented in hippocampus. Specifically, the increase in Ca²⁺ rises
426 triggered by somatosensory stimulation in rat hippocampal astrocytes is mediated by cholinergic
427 neurotransmission, since it is blocked by the cholinergic inhibitor atropine (Navarrete et al., 2012).
428 Astrocyte activation, in turn, induces the long-term potentiation (LTP) of field EPSPs in CA3-CA1
429 synapses (Navarrete et al., 2012). These data support the notion that, in addition to setting circuit
430 dynamics for attention in sensory processing, cholinergic neuromodulation participates in the encoding
431 of new information during memory formation (Hasselmo & McGaughy, 2004). The importance of
432 neuromodulation *via* astrocytic Ca²⁺ in sensory cortical processing has also been reported for the *locus*
433 *coeruleus* (Ding et al., 2013) (Paukert et al., 2014) (Srinivasan et al., 2015). This brain-stem nucleus

434 amplifies as well the effect of locomotion on Ca^{2+} rises in Bergman glia in the cerebellum (Paukert et al.,
435 2014). Timewise, neuromodulation-elicited Ca^{2+} rises in astrocytes occur in the range of a few seconds,
436 with regards to both onset and peak after sensory stimulation (Ding et al., 2013) (Srinivasan et al., 2015).

437 *5.2. Modulation of behavior and brain state by optogenetic and chemogenetic stimulation of astrocytes*

438 As in neurons, important insights into *causal* relationships between astrocytic Ca^{2+} signals and behavioral
439 outcomes are emerging from optogenetics and chemogenetic studies. These technologies allow
440 temporally-precise and reversible modulation of astrocyte activity, in contrast to permanent loss- or gain-
441 of-function genetic manipulations. In mice, optogenetic stimulation of astrocytes using ChR1/2, Arch and
442 OptoGq has been reported to modulate breathing according to pH changes in the respiratory system
443 (Gourine et al., 2010), induce long-term depression in Purkinje cells and motor behavior (Sasaki et al.,
444 2012), modulate response selectivity of the visual cortex (Perea et al., 2016), inhibit food intake (Sweeney
445 et al., 2016), induce sleep (Pelluru et al., 2016), promote a switch to the slow-oscillation state by triggering
446 the UP state of slow waves (Poskanzer & Yuste, 2016), and enhance memory acquisition (Adamsky et al.,
447 2018).

448 A key issue is that the downstream consequences of optogenetic activation of astrocytes are not well
449 understood. In the case of neurons, since they are excitable cells that can operate *via* all-or-nothing
450 changes in membrane voltage driven by fast-acting voltage-gated channels (although they also have
451 subthreshold voltage fluctuations), the probability of neuronal firing is decreased by activation of NpHR
452 and Arch, and increased upon activation of ChR2 (Yizhar et al., 2011). However, astrocytes are not as
453 electrically excitable as neurons. In the first report of successful modulation of neuronal activation (with
454 no behavioral consequences) upon optogenetic manipulation of nearby ChR2-expressing astrocytes, it
455 was assumed, but not shown, that the response was mediated by Ca^{2+} fluxes through ChR2 (Gradinaru et
456 al., 2009). Two subsequent studies confirmed Ca^{2+} rises using Ca^{2+} indicator dyes (Perea et al., 2014)
457 (Pelluru et al., 2016), yet it is unclear how these rises can occur, considering that ChR2 has a relatively
458 low Ca^{2+} permeability, is only open during a few milliseconds —decay constant is ~10 ms—, and presents
459 depolarization-dependent slowing of deactivation (Nagel et al., 2003; Yizhar et al., 2011). One possibility
460 is that it is the entry of Na^+ through ChR2 that causes Ca^{2+} uptake by reverse activity of the Na^+/Ca^+
461 exchanger (J. Yang et al., 2015). Further, the possibility exists that the effects of ChR2 activation are due
462 to undetected Ca^{2+} rises in astrocyte processes, of which somatic Ca^{2+} might be a consequence
463 (Bernardinelli et al., 2011). In this regard, the use of Arch combined with genetically-encoded Ca^{2+}
464 indicators represents a technical refinement because this opsin induces, after 5 s of photo-stimulation in
465 the mouse cortex, fast Ca^{2+} transients in astrocyte arbors reminiscent of spontaneous activity (Poskanzer
466 & Yuste, 2016). Still, how such a brief photo-stimulation of Arch, whose decay constant is ~9 ms (Yizhar
467 et al., 2011), translates into ~20-s-long Ca^{2+} rises after a delay of ~10 s is unclear (Poskanzer & Yuste,
468 2016). Plausibly, Arch-elicited hyperpolarization engages voltage-sensitive elements in astrocyte
469 processes. All in all, optogenetics clearly activates astrocytes, although clarification of underlying
470 mechanisms will help optimize this approach for Systems-level basic and clinical studies.

471 A DREADD receptor that successfully triggers Ca^{2+} transients in astrocytes is hM3Dq (Bonder &
472 McCarthy, 2014; Chen et al., 2016). Studies using hM3Dq in astrocytes have shown: (i) changes in
473 neuronal activity, either reduced or increased firing, in the mouse arcuate nucleus with opposing effects
474 on feeding behavior, perhaps stemming from CNO dose differences, which, in turn, might launch complex
475 feedback loops leading to paradoxical data (Chen et al., 2016; L. Yang et al., 2015), (ii) regulation of

476 excitatory and inhibitory neurotransmission in the amygdala, with a net effect of reduced fear expression
477 in a fear-conditioning paradigm (Martin-Fernandez et al., 2017); and (iii) potentiation of the amplitude
478 of evoked EPSC and, when chemogenetic activation is carried out at specific stages during learning
479 paradigms, improvement of contextual and spatial memory acquisition (Adamsky et al., 2018). As with
480 optogenetics, caution has to be exerted about the resemblance of the Ca^{2+} signaling elicited by
481 chemogenetics to physiological signaling. Also, the CNO metabolite clozapine, and not CNO, might be the
482 real activator of DREADD, as shown with radioligand receptor occupancy measurement, and *in vivo*
483 positron emission tomography (Gomez et al., 2017). Since clozapine has multiple targets, this recent
484 evidence raises doubts about the specificity of DREADD-based approaches (Gomez et al., 2017). That
485 said, these studies offer several computational insights, to be discussed below.

6. Computational lessons learned from Systems-like studies in astrocytes

487 *First, time scales of Ca²⁺ responses and filtering effect*. According to Ca^{2+}-based dynamics, the time scale
488 of astrocyte activation after a physiological input ranges from hundreds of milliseconds to tens of seconds,
489 while the earliest reported effect on nearby neurons after optogenetic stimulation of astrocytes is at 500
490 ms (Gourine et al., 2010). The onset of hemodynamic response is within 1-3 s from the onset of Ca^{2+}
491 responses (Otsu et al., 2015). Upon sensory stimulation, astrocytes are activated *after* neurons in cortex,
492 suggesting that neurons *reroute* information to astrocytes. The observation that Ca^{2+} response curves in
493 astrocytes are qualitatively similar but narrower than those in neurons, as shown by local field potentials
494 (Schummers et al., 2008; X. Wang et al., 2006), suggests that astrocytes *filter* neuronal activity. Filtering
495 can be either in terms of rectification (high pass filtering), cut-off (low pass filtering) or both (band pass
496 filtering). The latter appears to be the case since astrocytes are not responsive to the highest and lowest
497 frequencies of neuronal input. Interestingly, adaptive modulation of breathing by pH is the only context
498 in which astrocytes *directly* compute external stimuli, for astrocytes sense changes in pH, even if local
499 neurons are inactivated with tetrodotoxin (Gourine et al., 2010). In other paradigms, astrocyte activation
500 is either secondary to neuronal activation (section 5.1), or the result of gain-of-function induced by
501 optogenetics and chemogenetics in the context of already active circuits (section 5.2).

502 *Second, existence of short- and long-term modalities in Ca²⁺ responses*. The computational and
503 homeostatic functions of astrocytes manifest themselves in at least two broad modalities, depending on
504 time range, nature of inputs, and the intracellular location of Ca^{2+} rises. One modality is the fast rising
505 Ca^{2+} signals that originate within 0.2–5 s from stimulus onset, are short-lived (up from 0.3–10 s), are
506 usually reported in peripheral processes and end-feet (e.g., (Stobart et al., 2018), and are sufficiently fast
507 to *locally* mediate task-relevant regulation of blood flow (Otsu et al., 2015), metabolic coupling, and
508 neurotransmitter supply (Agarwal et al., 2017; Otsu et al., 2015; Tani et al., 2014), as well as short-term
509 modulation of synaptic efficacy (Perea et al., 2016). The second modality corresponds to robust somatic
510 Ca^{2+} transients that can last tens of seconds, have a slow rise time, and have been reported in the context
511 of cholinergic (Navarrete et al., 2012; Takata et al., 2011) and noradrenergic (Ding et al., 2013) (Paukert
512 et al., 2014) (Srinivasan et al., 2015) neuromodulation, as well as upon ChR2-based optogenetics and by
513 chemogenetics (Adamsky et al., 2018). In hippocampus, the functional consequences of this modality are
514 long-lasting effects on synaptic connections (Adamsky et al., 2018; Navarrete et al., 2012), plausibly
515 associated with memory formation. In cortex, we reason that astrocytic Ca^{2+} rises, as reported by (Takata
516 et al., 2011), participate in a well-accepted role of neuromodulation: control of arousal and attention,
517 which involves recruitment of large, spatially-distributed neuronal populations (Thiele & Bellgrove,
518 2018). Importantly, the two modalities reveal the existence of *threshold heterogeneity* in Ca^{2+} responses

519 in astrocytes, which might be of computational importance. Consider, for example, the relative ease with
520 which minimal synaptic stimuli trigger Ca^{2+} transients in astrocytic processes (Haustein et al., 2014;
521 Panatier et al., 2011), which is consistent with a relatively low threshold for activation. This suggests that,
522 in microdomains, the number of synaptic inputs may be of little importance, so that a microdomain could
523 invariantly get activated, either by individual synapses or by an ensemble thereof, akin to the logical OR
524 function. Conversely, the phenomenon of *coincidence detection* in which activation of cortical sensory
525 neurons (Paukert et al., 2014; Takata et al., 2011) and postsynaptic hippocampal neurons (Navarrete et
526 al., 2012), needs to coincide with neuromodulation to trigger somatic Ca^{2+} transients, and, similarly, the
527 requirement for high inter-neuronal activity to promote astrocytic Ca^{2+}-dependent facilitation of
528 excitatory synaptic transmission in the hippocampus (Perea et al., 2016), may be regarded as examples
529 in which the threshold for astrocytic activation is high, and astrocytes will become activated only if
530 multiple inputs impinge *together* on them, akin to the logical AND function. Density of IP3R2 (De Pitta
531 et al., 2018) and baseline Ca^{2+} levels (Zheng et al., 2015) may be among the factors setting thresholds of
532 stimulation. Plausibly, the described modalities of astrocytic Ca^{2+} responses are the extremes of a context-
533 dependent spectrum, encompassing mixed regimes in terms of number of astrocytic domains involved,
534 and short *versus* long-term effects. Key questions emerge: how are different astrocytic microdomains
535 recruited, which neural circuits are activated as a consequence of different response modalities, and,
536 finally, do specific computations, other than thresholding, operate in different modalities? In section 7,
537 we propose gaining insight into these questions by treating single astrocytes as mini-circuits, and by
538 identifying relevant patterns of Ca^{2+} responses with dynamical-systems statistics approaches such as
539 *dimensionality reduction.*

540 *Third, regulation of neuronal gain.* This appears to be a computation carried out by astrocytes
541 throughout a variegated collection of circuits and behavioral contexts. Signal coincidence detection of
542 sensory stimulation and neuromodulation by cortical astrocytes is one example that may have
543 implications in attention (Paukert et al., 2014; Takata et al., 2011). Computationally, attention consists of
544 a gain change (in amplitude of response or contrast) that results in the prioritization of relevant inputs
545 over irrelevant information (Thiele & Bellgrove, 2018). Input prioritization is called top-down (or inside-
546 out) because the process is shaped by internal models and goals conveyed to the sensory areas by
547 neuromodulators (Thiele & Bellgrove, 2018)—note the influence of predictive coding in this assumption.
548 The modulation of gain is facilitated by a normalization mechanism whereby neurons' responses are
549 reduced in proportion to the activity of neighboring neurons by the joint activation of inhibitory and
550 excitatory neurons (Reynolds & Heeger, 2009). Instructed by signal coincidence detection, astrocytes
551 might help prioritize information by regulation of gain *via* modulation of excitatory synaptic drive by
552 Ca^{2+}-dependent glutamate uptake (Schummers et al., 2008), gliotransmission (Takata et al., 2011),
553 intrinsic neuronal excitability (Sasaki et al., 2012), and co-modulation of excitatory and inhibitory
554 neurotransmission (Perea et al., 2014).

555 In the case of brain state, a gain change might account for the transition from an asynchronous to a
556 synchronous mode through a change in the network's ratio of excitation *versus* inhibition, according to
557 the general theory of neural networks (Brunel, 2000). Hence, a possible mechanism whereby astrocytes
558 might synchronize brain state through gain control is regulation of excitatory-synaptic strength, either by
559 reducing glutamate uptake (Poskanzer & Yuste, 2016), releasing ATP/adenosine and glutamate in a Ca^{2+}
560 dependent manner (Halassa et al., 2009) (Fellin et al., 2009), or taking up GABA *via* GAT-3 transporters
561 (Shigetomi et al., 2011).

Memory-related tasks in hippocampus can also be interpreted as a phenomenon of gain control. Thus, chemogenetic and optogenetic stimulations of hippocampal astrocytes result in increased frequency and potency of mEPSCs in local neurons, leading to long-term potentiation of excitatory synaptic connections (Adamsky et al., 2018). Significantly, astrocyte-mediated NMDA-dependent long-term potentiation appears to be: (i) task-specific insofar as fear-conditioned mice, but not home-caged ones, show synaptic potentiation, and (ii) stage-selective, for it very precisely affects distinct phases along the memory-formation continuum, such as memory allocation. Likewise, the interneuron-induced potentiation of excitatory neurotransmission mediated by astrocytes might be one example of neuronal gain (Perea et al., 2016). Intriguingly, a dual mechanism in which astrocyte-mediated depression of excitatory synapses combines with potentiation of inhibitory ones seems at play in afferents to neurons in the medial central region of the amygdala (Martin-Fernandez et al., 2017). The ensuing net increase of inhibitory drive to these neurons (i.e., a case of negative gain) was then shown to correlate with transient reduction of fear conditioning and anxiety

Finally, the role of astrocytes in reflex homeostatic behaviors modulating feeding and breathing can be explained in terms of use of gain modulation to adapt behavior to stimuli intensity. Thus, the presence of food modulates the synaptic efficacy of neurons in the hypothalamus (Chen et al., 2016; L. Yang et al., 2015), whereas pH acidification induces adaptive neuronal firing in the brain stem which, in turn, activates breathing (Gourine et al., 2010).

Fourth, decoding and rerouting of information. Coincidence detection of sensory cortical and neuromodulatory subcortical neuronal inputs (Takata et al., 2011) (Paukert et al., 2014), transformation of inhibitory neurotransmission into synaptic facilitation in hippocampus (Perea et al., 2016), and the transformation of neuronal inputs into potentiation or inhibition, depending on the duration and frequency of the inputs (Covelo & Araque, 2018), might be three examples of *decoding* of neuronal signals by astrocytes, and *rerouting* of decoded information to other neurons. Plausibly, the information rerouted by astrocytes is gliotransmitter-dependent (Covelo & Araque, 2018). Since neuronal action potentials and astrocytic Ca^{2+} transients have utterly different temporal resolutions, it is improbable that variables represented in trains of action potentials are represented in astrocytic Ca^{2+} without significant loss of information. Rather, we posit that what astrocytes 'hear' from neurons are instructions to 'tell' other neurons to modify their activity *via* canonical computations. In computational science, canonical computations are fundamental operations carried out in circuits in a variety of contexts. We have hitherto identified a few: signal filtration, thresholding (implicating AND/OR functions and coincidence detection), gain, and control of the balance between excitation and inhibition. It is not clear whether synaptic scaling should be added, because this function might be performed by microglia rather than astrocytes (Stellwagen & Malenka, 2006). In the roadmap we propose to use *decoding approaches* from machine learning to identify possible variables encoded by astrocyte computations.

Fifth, astrocytes could act as switches in brain state transitions. The causal implication of astrocytes in cortical slow oscillations (<1 Hz) (Takata et al., 2011) (Poskanzer & Yuste, 2016) supports the relevance of astrocytes in network activity beyond tripartite synapses. Slow waves have been hypothesized to represent the default mode of cortical network activity (Sanchez-Vives et al., 2017). During UP states, there is synchronization in beta and gamma frequencies, synaptic gain modulation, modulation of replay and memory formation, and some cortical features might inform about transitions between unconsciousness and consciousness (reviewed in (Sanchez-Vives et al., 2017)). An intriguing paradox

exists in that astrocytes induce a synchronized state, but also mediate cholinergic and noradrenergic neuromodulations, which are characteristically associated with asynchronous, high-rate activity that facilitates sensory processing (Lee & Dan, 2012). We posit that astrocytes might act as *switches* whose default action is to sustain UP states, whereas neuromodulation-driven attention renders astrocytes independent of the cortical oscillator, and shifts their action towards short-term plasticity related to sensory processing. Indeed, network theory predicts that a key parameter in setting asynchronous *versus* synchronous network activity, as well as the frequency of eventual oscillations, is afferent synaptic activity (Brunel, 2000; Ledoux & Brunel, 2011). Coincidence detection can be thus regarded as a scenario of afferent stimulation—specifically mediated by neuromodulation—whereby astrocytes induce the network's transition to the asynchronous state. Finally, although astrocytes are particularly attuned to slow oscillations because their internal dynamics, as judged by Ca^{2+} transients, fall within a time scale of seconds, they are also involved in the generation of faster waves such as theta (4–12 Hz) and slow gamma (30-50 Hz) (Perea et al., 2016; Sardinha et al., 2017). The effect of astrocytes on fast waves may be due to cross-frequency coupling, a mechanism whereby global slow oscillations modulate local fast oscillations, usually their amplitude (Canolty & Knight, 2010), which happens to be the predominant effect of astrocytes on fast waves (Perea et al., 2016; Sardinha et al., 2017). By regulating fast waves, astrocytes will have an impact on neuronal encoding, because fast rhythms provide temporal reference frames for local and large-scale computations (Hawellek et al., 2016). Dimensionality reduction (below) may reveal specific astrocytic Ca^{2+} regimes associated with coincidence detection, oscillations, and brain state transitions.

7. **A roadmap to advance the integration of astrocytes into Systems Neuroscience**

7.1.   Theoretical and conceptual improvements

Is there a minimal astrocyte-neuronal circuit? Anatomical, molecular and functional factors matter when considering astrocytes from a computational point of view. From an anatomical perspective, a single astrocyte can be regarded by itself as a 'mini-circuit', in light of the subcellular compartmentalization of calcium signals (Bazargani & Attwell, 2016), along with the consideration that one astrocytic anatomical domain may comprise numerous neurons, dendrites and synapses. Estimations in the mouse hippocampus are: 1-20 neurons (Halassa et al., 2007), 300-600 dendrites (Halassa et al., 2007), and 140,000 synapses in (Bushong et al., 2002) and 50,700-75,200 in (Chai et al., 2017). Recently, a FRET-based study reports dynamic interactions of astrocytic distal processes with different types of synaptic inputs (Octeau et al., 2018). Moreover, because astrocytes are characteristically territorial, they give rise to a tiled arrangement of the brain space, which can be then seen as a patchwork of mini-circuits. The function of tiling is an outstanding question. From a molecular perspective, according to single-cell gene profiling, and unbiased hierarchical clustering in mouse brains, astrocyte populations are not as functionally heterogeneous as neuronal populations (Zeisel et al., 2015). Thus, in the mouse somatosensory cortex and hippocampal CA1 region, there are 29 types of neurons including pyramidal cells, glutamatergic neurons, and interneurons, as opposed to just two types of astrocytes (Zeisel et al., 2015). This suggests that, although both neurons and astrocytes are molecularly specialized cells, additional and extensive sub-specialization exists among neurons but not astrocytes. On the other hand, the lack of molecular definition may provide astrocytes with greater adaptive capacity to operate in a variety of circuits (Poskanzer & Molofsky, 2018), which may explain phenotypical differences of astrocytes from region to region (Martin et al., 2015) (Chai et al., 2017). We thus argue that neurons imprint functional signatures on networks by encoding, for example, odors, position, images, words,

647 abstract categories and executive functions, whereas the size, anatomical arrangement and molecular
648 makeup of astrocytes suggest that they might be designed to operate canonical computations (Section 6,
649 Table 1) in local mini-circuits within larger-scale networks—as well as homeostatic and metabolic
650 support. Support for the hypothesis that astrocytes perform canonical computations comes from studies
651 showing that astrocyte-based computations such as synaptic potentiation, a type of gain control, improve
652 the performance of ANNs (Alvarellos-Gonzalez et al., 2012; Porto-Pazos et al., 2011). Additional support
653 comes from recent theoretical studies in computer science, and formal language theory, which showed
654 that canonical filtering of synaptic transmission by astrocytes (described as 'astrocyte-like control')
655 facilitates the generation of the so-called logic gates (Song et al., 2017), which are basic building blocks in
656 neural circuits performing logic Boolean operations such as AND, OR, NOT, XOR and NAND (Binder et
657 al., 2007). According to these studies, simple ensembles of astrocytes and synapses reminiscent of our
658 mini-circuits might account for all elementary logical functions and, properly combined, allow, in
659 principle, computation of any real-world function in a scalable manner (Song et al., 2017). It should be
660 kept in mind that multiple strategies are likely at play across species in shaping astrocytic mini-circuits,
661 and their possible computational functions. For example, although single-cell genomics is not yet
662 available in humans, the fact that human astrocytes are larger, more complex (including 270,000-2
663 million synapses), and present more morphological variants than mouse astrocytes (Oberheim et al.,
664 2009), together with the striking observation that engraftment of human astrocytes into mouse brains
665 enhances synaptic plasticity and learning (Han et al., 2013), suggests that more complex astrocytic mini-
666 circuits are present in humans, possibly underpinning a larger variety of canonical computations. All in
667 all, it appears that in order to reinforce the presence of astrocytes in Systems Neuroscience, we must zoom
668 out at astrocyte populations as well as zoom into single-astrocyte mini-circuits. This is akin to neuron-
669 focused studies that, as noted, should cover both systems-wide and sub-cellular computations. Indeed,
670 the latter should be considered as part of the computations within astrocyte mini-circuits, for spines and
671 dendrites are inextricably embedded in an astrocyte 'matrix'.

673 *Where might the 'slow' spatiotemporal dynamics of astrocytic Ca²⁺ enter Systems Neuroscience?* The
674 question of which time scales are relevant for neuronal computations has long been debated. Action
675 potentials of individual neurons are characteristically fast and short-lived voltage depolarizations in the
676 range of 1-2 ms. The speed and all-or-nothing nature of these responses, as well as their lack of
677 attenuation due to axonal myelination, makes them well suited to transmitting information throughout
678 the brain in milliseconds. Currently, the *minimal* temporal resolution of the neuronal code appears to be
679 on a millisecond time scale, as shown in sensory processing in the auditory system of mammals (Butts et
680 al., 2007) (Kayser et al., 2010), and in basic human cognitive capabilities, including semantic abstract
681 categorization of images (e.g., identifying an image as a 'dog')(Vanmarcke et al., 2016). This means that
682 stimuli arriving within intervals of a few milliseconds are distinguished as individual entities by neurons
683 that fire individual, millisecond-long spikes in response to each stimulus. Clearly, if astrocyte Ca²⁺
684 transients are the astrocytic substrate of neural computing—and they are the best candidate thus far—
685 they are too slow to encode ultrafast representations. However, the brain characteristically operates in
686 parallel on a gradient of time scales that are nested and hierarchically organized (Murray et al., 2014).
687 Thus, attention and decision making can last seconds, emotions can arise within seconds, and mood
688 changes in minutes. In prediction coding, the slow contextual changes in the prefrontal brain under which
689 fast sensory representations are interpreted require seconds (Kiebel et al., 2008). Also, there are
690 circadian time scales affecting sleep and global homeostasis, and very long time scales in the range of
691 hours, weeks, or years affecting learning and memory (Hari & Parkkonen, 2015). This means that,

complex operations ought to exist prolonging the effect of ultrafast (up to 10 ms) and fast (<100 ms) neuronal time scales up to minutes, which precludes structural changes caused by gene expression. Working memory during decision making is a prototypical example of the need for sustained activity in the short-term scale. The question is how several discrete, millisecond-long events related are engaged in a continuum of network activities that last up to hundreds of seconds (Hasson et al., 2015). Since there is no external input during delays (time between input and action), working memory must arise from the intrinsic dynamics of neural circuits. Computational neuroscience identified this problem over 20 years ago (Seung, 1996), and has since struggled to provide answers using realistic neuronal parameters (Chaudhury and Fiete, 2016). Answers include: (i) biophysical properties of neurons such as the slow 'membrane-time constant', which reflects the time during which information can be maintained by neuronal voltage without a substantial leak, estimated to last between 5-20 ms, (ii) intervention of NMDA receptors, which are ideally suited to enlarge 'memory' capabilities of neurons beyond their membrane time constants because they are active around 100 ms after the synaptic input (X. J. Wang, 1999), (iii) short-term synaptic plasticity (Abbott & Regehr, 2004), (iv) an effective computational solution called long short-term memory (Hochreiter & Schmidhuber, 1997), and (v) sustained firing rate of neurons, or 'persistent activity', achieved upon the exquisite tuning of recurrent circuits such that an input re-entering a synapse exactly matches the decay of the neuron, keeping its firing rate for a prolonged time (Goldman-Rakic, 1995) (Renart et al., 2007). These solutions present limitations. Slow time constants need to be reset, and, at present, slow time constants in neurons do not seem to have that capability. The time constant of the NMDA receptor is appropriate to maintain memories up to 1-5 s, but not longer. Long short-term memory works very well in current machine learning applications, but its application to natural circuits is unclear. Finally, it is also unclear how the exact timing of feedback loops in persistent activity is achieved. Clearly, additional solutions are in order, perhaps including astrocytes.

Inclusion of astrocytes in current theoretical frameworks and circuit-operating principles. The temporal dynamics of Ca^{2+}-based excitability make astrocytes suitable to operate in circuit computations running in the sub-second to a supra-second scale, including the ones already mentioned such as short-term plasticity, neuromodulation, and slow rhythms. Interestingly, computations such as signal-coincidence detection and oscillation control imply detection of the order and interval of arrival of time-varying signals, suggesting that astrocytes might encode time. Theoretical models of timing in the brain such as oscillators (Goel & Buonomano, 2014) and liquid state (or liquid computing) (Maass et al., 2002) may be useful to explore this idea. Astrocytes might also have a role in predictive coding. As shown *in silico* renditions (Deneve et al., 2017), the core idea of the framework is that neural circuits are error-driven, such that differences between predictions and new inputs are computed as prediction errors, which might be transformed (i.e., 'rerouted') into changes in synaptic strength by short-term plasticity. The greater the error, the more synaptic changes would be needed in order to 'update' circuit information. The quality of prediction errors is computed by the variable 'precision', which is akin to the standard error in the t-Student test, and is hypothesized to occur in a scale of seconds, and to be encoded by neuromodulators (Friston, 2009; Stephan et al., 2015). Since astrocytes participate in neuromodulation (Navarrete et al., 2012; Takata et al., 2011) (Ding et al., 2013) (Paukert et al., 2014), the possibility emerges that astrocytes might encode precision, perhaps by temporally decoding prediction errors from multiple synapses in the astrocyte mini-circuit, in order to ensure sufficient statistics. It is tempting to speculate that the aforementioned canonical computations carried out by astrocytes are manifestations of computation of error-related statistics and/or time in different contexts. These computations would be canonical, for they would occur throughout the brain. Decoding analyses (below) may provide

736 information about the specific computations carried out by astrocytes in complex behaviors where issues
737 like timing, temporal holding of information, and error between predictions and real outcomes, are
738 particularly prominent.

739 *Astrocytes and energy-efficient coding.* Circuit modeling and biophysical analyses support the idea that
740 neuronal circuits are designed to produce energy-efficient codes because action potentials are
741 energetically demanding; hence, energy supply becomes a relevant constraint in information processing
742 (Laughlin, 2001). Three reasons justify a revision of the adjustment of coding to energy constraints from
743 the perspective of astrocytes. *First*, astrocytes may lessen the metabolic constraint by facilitating lactate
744 to neurons during task-elicited glutamatergic neurotransmission (Magistretti & Allaman, 2015). Of note,
745 lactate qualifies as a gliotransmitter, and hence may be harvested for computational signaling tasks,
746 because it instructs memory acquisition (Suzuki et al., 2011), and stimulates neurons by a mechanism
747 independent of its uptake, perhaps receptor-mediated (Tang et al., 2014). *Second*, as noted in (Magistretti
748 & Allaman, 2015), the anatomical arrangement of local neurons, projections from neuromodulatory
749 nuclei and astrocytes within cortical columns, points to optimized circuit design to facilitate energetic
750 coupling between neurons and astrocytes. Here we extend this notion to astrocyte mini-circuits, and
751 argue that they might represent a coding strategy to optimize energy utilization, for example, by
752 integrating sparse coding, which is coding distributed among many synapses to reduce individual
753 computational load, and has been described as a solution to energy limitations (Laughlin, 2001). *Third*,
754 whether energy is also a constraint in Ca^{2+}based computations in astrocytes is an outstanding question.
755 There is currently no estimation of the energy demand of Ca^{2+}signaling in astrocytes. ATP-consuming
756 steps are: (i) in the context of IP3R2-mediated Ca^{2+}-release, re-uptake of cytosolic Ca^{2+} back into the
757 endoplasmic reticulum *via* Ca^{2+}/ATPase pumps, which are crucial in dictating the period of Ca^{2+}
758 fluctuations/oscillations, as well as their shape and duration; (ii) the plasmalemma Ca^{2+}/ATPase pump
759 involved in capacitive Ca^{2+} entry/flux; (iii) Na^+/K^+-ATPase activity dependent on glutamate uptake
760 (Pellerin & Magistretti, 1997), which appears to critically influence Ca^{2+} rises in sensory processing
761 (Schummer et al., 2018); (iv) V-ATPase dependent uptake of Ca^{2+} into acidic stores; and (v) neuronal-
762 activity dependent Ca^{2+} rises in astrocytic microdomains in distal processes, as shown in mice with
763 membrane-anchored GCaMP3 (Agarwal et al., 2017). This study documents a critical link between energy
764 metabolism and Ca^{2+}-based excitability, because it shows that Ca^{2+} rises in microdomains are the result
765 of Ca^{2+} efflux from mitochondria, which, in turn, is triggered by short events ('mitoflashes') of superoxide
766 production during oxidative phosphorylation. Still, the need for ATP for several critical processes is an
767 open question, a prime example of which is gliotransmission: the exact source of gliotransmitters such as
768 ATP, glutamate, and D-serine, and the energy expenditure involved in their production, is unknown. All
769 in all, it is worth stressing that fatty acids are a fuel for oxidative metabolism in astrocytes (Eraso-Pichot
770 et al., 2018). Since fatty-acid oxidation yields over 50 times more ATP molecules than glycolysis, astrocyte
771 metabolism might be optimized to undertake costly computations from the point of view of energy
772 requirements.

773
774 *Ca^{2+}-independent computations.* Although productive, the adoption of Ca^{2+} signaling as a readout of
775 astrocyte excitability should not blind us to the possibility that, similar to Ca^{2+} transients in neurons
776 following action potentials, the astrocytic Ca^{2+} response might be a late manifestation of yet undiscovered
777 signals. If we recover classic perspectives of biophysics (Barlow, 1996; Destexhe, 1999), many
778 components of the astrocytic response could potentially encode stimulations and perform computations.
779 This is the case of second messenger molecules such as IP_3 or cAMP that are conventionally associated

780 with GPCR-mediated astrocytic Ca²⁺ signaling (DePittà, 2019) but also other ion-based signals. Among
781 the latter, Na⁺ is an emerging candidate because it presents activity-dependent fluctuations, although
782 advanced fluorescent probes are necessary to fully establish this ion as a novel readout of astrocyte
783 excitability (Rose & Verkhratsky, 2016).

784 **7.2. *Technical and analytical improvements***

785

786 *7.2.1 Zooming into astrocyte mini-circuits*

787

788 *Dimensionality reduction of Ca²⁺ data.* We posit that single-astrocytes and astrocyte populations are
789 dynamical systems governed by function-specific regimes resulting from coordinated changes in Ca²⁺
790 signaling. At the single-astrocyte level, the local and global activation modalities described earlier might
791 be the extremes of a spectrum of possible regimes. Dimensionality reduction is a statistical method
792 developed in machine learning to facilitate analysis of the characteristically multidimensional (i.e.,
793 multivariate) dynamical systems. What dimensionality reduction does is to identify key variables
794 determining relationships within the data (the so-called latent variables), thereby reducing input data to
795 low-dimensional representations defined by such latent variables. In Systems, dimensionality reduction
796 has been applied to neuron-population recordings in decision making, movement, odor perception,
797 working memory, visual attention, audition, rule learning, and speech (reviewed in (Cunningham & Yu,
798 2014). The complex spatiotemporal patterns of spontaneous and evoked Ca²⁺ transients in single
799 astrocytes, which now can be measured with 3-dimensional Ca²⁺-imaging (Bindocci et al., 2017),
800 represent a multidimensional data set that will benefit from dimensionality reduction techniques. Thus
801 far, Ca²⁺ transients in astrocytes have been simplified for quantification purposes by using a single Ca²⁺
802 readout (Perea et al., 2014), the average of calcium signals detected in multiple ROIs pooled from a
803 population of astrocytes (Poskanzer & Yuste, 2016), the categorization of these signals by spatial location
804 and averaging within subcellular compartments (Chai et al., 2017), and machine-learning based
805 identification of true signals (Agarwal et al., 2017). Although these approaches have already yielded useful
806 insights into correlations between astrocytic and neuronal activities and behaviors—as described in
807 Section 6—they have not revealed possible canonical spatiotemporal computations within and between
808 astrocytes, in distinct experimental paradigms. Dimensionality reduction will thus facilitate detection of
809 noise (stochastic Ca²⁺ transients), indicate whether some of the manually selected ROIs based on visual
810 inspection are not independent, and hence can be considered as the same ROI, and reveal correlations
811 and anti-correlations of distant regions belonging to the same ROI. The latter can occur when distant
812 regions are synchronized due to oscillations or synchronous inputs that regularly occur in those regions.
813 Thus, dimensionality reduction of single-astrocytes may help to reveal and select dimensions, that is, the
814 minimum number of ROIs (e.g., 5-10 from up to 200 original ones), in which fluctuations are more
815 pronounced and meaningful, thus paving the way for population analyses, which will require the
816 simplification of Ca²⁺ signals per astrocyte with the minimal loss of relevant information. Linear methods
817 for dimensionality reduction that can be used in astrocytes include simple principal component analysis
818 (PCA), the prime linear method (Cunningham & Yu, 2014), as well as factor analysis, as used with
819 neuronal Ca²⁺ (Paninski & Cunningham, 2018).

820

821 *Machine learning.* Non-linear methods such as ANNs are increasingly being used to replace stages in
822 signal processing and analysis in neuronal populations, as well as a method for dimensionality reduction
823 (Paninski & Cunningham, 2018). Thus, ANNs could *a priori* uncover latent variables that best account

824 for Ca^{2+} data from astrocyte mini-circuits, and are non-linearly related. Current ANNs appear well-suited
825 to extract latent variables from Ca^{2+} imaging of large populations of neurons (Paninski & Cunningham,
826 2018), and their application to multidimensional astrocytic Ca^{2+} data should be explored. Conversely,
827 ANNs can be also used as generative models, that is, models that infer classes of inputs from a low number
828 of latent variables (Dosovitskiy, 2015). Another statistical tool of machine learning that holds promise is
829 Bayesian hierarchical modeling (Bishop, 2006). The general idea is to build a graph that hierarchically
830 and probabilistically relates relevant variables related to Ca^{2+} and to other data from connectomics.
831 Indeed, if the graphs are well-informed about the connectome within mini-circuits, they can be used as
832 an inverted model to infer the values of the latent variables accounting for Ca^{2+} signals. One advantage of
833 these methods is that the number of free parameters is typically lower than in standard ANNs, which
834 might require massive amounts of data for training.

835 *Connectomics*. Providing an accurate picture of the synaptic contacts within astrocyte mini-circuits, in
836 rodents and humans, and in different brain regions, is necessary to help interpret and model *in*
837 *silico* Ca^{2+}-based regimes defined by dimensionality reduction, and to identify constraints that could be
838 incorporated into machine-learning algorithms. Specific questions are the density of excitatory and
839 inhibitory synapses (and subtypes of the latter), their functional interplay in distinct astrocyte regimes
840 defined by Ca^{2+}. For example, astrocyte mini-circuits might adopt feed-forward, recurrent or mixed
841 patterns, depending on the behavioral task, and present hierarchical organizations between astrocytic
842 and neuronal elements, as well as topological/functional 'motifs' and wiring rules—as shown in the
843 analysis of small neuronal networks (Schroter et al., 2017). Tools for connectomics include graph theory
844 (Fornito, 2016), Bayesian hierarchical modeling (Bishop, 2006), and topological tools (Kanari et al.,
845 2018; Reimann et al., 2017). In all these approaches, both morphological and functional readouts could
846 serve as input data. Morphological readouts of the synaptic architecture of astrocyte mini-circuits at
847 meso- and micro-scales can be obtained with array tomography, a form of light microscopy based on the
848 serial sectioning of ultrathin (hundreds of microns) sections, which permits 3D reconstructions at a
849 micrometer/nanometer resolution (Micheva et al., 2010). Array tomography can be complemented with
850 automated 3D electron microscopy techniques, such as serial block-face ANNs electron microscopy
851 (SBFSEM). Crucially, fixation methods must not distort contacts within mini-circuits (Korogod et al.,
852 2015). Functional analyses are more challenging, for they will require development of improved optical
853 tools and probes to simultaneously monitor the activities of excitatory and inhibitory neuronal
854 populations, as well as those of astrocytes. The emerging combination of 2-photon calcium imaging with
855 SBFSEM for examining neural circuits at cellular resolution may pave the way for subcellular analyses
856 (Vishwanathan et al., 2017). Finally, recent multiplex Ca^{2+} imaging at a single synapse-astrocyte
857 interface (J. P. Reynolds et al., 2018), application of nanotechnology to voltage recording in
858 neurons (Jayant et al., 2017), and FRET-based analysis of contacts between synapses and astrocytes
859 (Octeau et al., 2018), are advances towards integrating structure and function in astrocyte mini-circuits.

860 *7.2.2. Zooming out to astrocyte populations*
861

862 *Decoding astrocytes in complex behavioral tasks*. The identification of a astrocytic Ca^{2+}-based code is a
863 prime objective that, importantly, can be started with current statistical tools developed to study neuron-
864 based encoding and decoding. Moreover, we argue that the increased interest in neuronal Ca^{2+} as a tool
865 to decipher the brain code (the reason being that the number of neurons recorded with optical tools is
866 one order of magnitude higher than with multi-electrode arrays, see Section 2) benefits the analysis of

Ca²⁺-based astrocyte computations. For simplicity, here we focus on decoding approaches, which specifically seek to predict external variables from signal patterns, although tools to study encoding can be also considered (Section 3). Decoding astrocyte signals entails measuring Ca²⁺ activity populations in behavioral paradigms in which several time scales, including those in the range of action defined for Ca²⁺-based signaling in astrocytes (hundreds of milliseconds to tens of seconds), are relevant for the task at hand. One such paradigm is reward-associated decision making over variable contexts in which an animal must associate stimuli with choices (responses) to obtain an immediate reward. The association can be abruptly reversed, as in the case of reversal learning, where in a given context 1, stimulus A leads to reward and stimulus B does not lead to reward, whereas in another context 2, stimulus B predicts reward (Schoenbaum et al., 2002). The performance in such varying contexts involves tracking variables at both fast and slow time scales. Variables such as 'immediate reward', 'confidence', 'option values' and 'choice' are fast, represented in the millisecond time scale, whereas the deliberation occurring before a decision is taken lasts hundreds of milliseconds to seconds, and even up to minutes if this deliberation involves inference about the current context. During this time, the brain computes correlations between fast variables, and represents differences between the prediction based on previous experience and the real outcome as 'error'. We argue that the precise computation of prediction error is key in the identification of a true association between stimulus and reward, such that varying contexts plausibly require more complex computations. Frontal areas are expected to track the mixture of relevant variables in the form of 'cognitive maps'. In rat, the orbitofrontal cortex encodes the millisecond-long fast variables (Rolls et al., 1996) (Nogueira et al., 2017). It is unclear, however, how transitions between contexts and associated deliberations are represented at the much slower time scale of seconds. We posit that the network may use astrocytes as a buffer to help represent the prior history of rewards and choices, which is necessary to infer the true nature of the current context. Specifically, astrocytes may temporally integrate error signals, or somehow influence behavior based on accumulated information through canonical computations such as gain modulation. Along these lines, dopaminergic neuromodulation, which signals reward prediction error (O'Doherty et al., 2017), might serve to gate information from neurons to astrocytes, and vice versa.

Technical and analytical challenges associated with large-scale recordings of Ca²⁺ rises in astrocytes and neurons. The specific experimental design we propose involves the simultaneous recording of Ca²⁺ activity in astrocytes with 2-photon microscopy in awake animals (Srinivasan et al., 2015), and Ca²⁺ or electrophysiological responses in neurons (Poskanzer & Yuste, 2016). From previous work indicating that with tens of neurons it is possible to predict animal choices with high accuracy (Kiani et al., 2014; Nogueira et al., 2017), we reason that tens of astrocytes will suffice to observe statistically significant trends that can be used to guide subsequent recordings and analyses. At this time, optimal selection of paradigms and analytical methods may be more helpful to make significant leaps towards understanding astrocyte-based computations than massively increasing the number of astrocytes recorded. Data acquisition, signal processing and increased dimensionality of the data present additional challenges when there is a need to perform recordings of two cell types with different Ca²⁺ dynamics. As to data acquisition, although recent advances have pushed the boundaries of multi-photon imaging, with significant improvements that enable imaging in multiple brain areas, across *laminae*, and in non-head-fixed configurations (Yang & Yuste, 2017), since these imaging methodologies have been developed specifically to record the activity of neuronal populations, they may not always be translatable to astrocyte populations. For example, many of the technologies used to carry out 3D two-photon imaging rely on source separation algorithms that assume the Ca²⁺ signals are non-propagative and spatially static. While

this is true for Ca²⁺ imaging of neuronal somata, astrocyte Ca²⁺ imaging data obviously do not obey these rules. Thus, new 2-photon imaging methodologies born from an astrocytic perspective, particularly those that allow imaging multiple *laminae* simultaneously, are necessary to advance our understanding of these cells within larger, meso-scale circuits. Another area of improvement for large-scale Ca²⁺ recording in astrocytes and spike-recording in neurons is the development of new electrophysiological approaches, including flexible polymer probes (Chung et al., 2018) and clear electrode arrays (Thunemann et al., 2018), to solve the current problem posed by the large equipment necessary to carry out single-neuron recordings, which precludes astrocyte imaging. Despite the advances in Ca²⁺ imaging, single-neuron electrophysiological measurements are preferable, for Ca²⁺ transients lack temporal resolution to reveal single-action potentials. With regards to signal processing, we described earlier the state-of the-art in signal processing in large-scale recordings in neurons, including methods to denoise, demix and simplify Ca²⁺ data. As to astrocytes, readouts to be assessed per astrocyte are Ca²⁺ signals in microdomains measured in dynamic ROIs (Wang et al., 2016) (Agarwal et al., 2017), and/or processed with dimensionality reduction techniques as explained above. *A priori*, dimensionality reduction and decoding techniques can be used with data from astrocyte *and* neuronal populations. Possible experimental scenarios are paired Ca²⁺ imaging from both cell types (e.g., low-dimensional data *per* astrocyte could be paired with one optical or electrophysiological signal *per* neuron). Dimensionality reduction may reveal pools of neurons interacting with specific astrocytes. Similarly, linear and non-linear decoders could be trained to predict relevant behavioral variables from neuron-astrocyte networks, and to study which sets of neurons and astrocytes are more relevant for that decoding. Linear decoding techniques could be used even if the amount of behavioral data is not massive, such that around ten trials per stimulus-choice condition might suffice to obtain a description of astrocyte-neuronal interactions at behaviorally relevant time scales.

7.3. Translation: Clinical Systems Neuroscience

When it comes to treatments for CNS diseases, molecular and cellular approaches should not be abandoned, because they have successfully led to current therapeutic venues. For example, in multiple sclerosis, relapses are mitigated by immunotherapy against specific populations of immune cells (Torkildsen et al., 2016), and in Alzheimer's disease, promising anti-β–amyloid treatments are being tested in clinical trials (K. V. Kastanenka et al., 2016; Sevigny et al., 2016). However, there are no effective preventive or disease-modifying treatments for neurodegenerative and psychiatric disorders, suggesting that reductionist approaches aimed at fighting disease one molecule or one cell at a time might be insufficient. Moreover, degeneration of neuromodulatory nuclei (Kelly et al., 2017; Liu et al., 2015), as well as large-scale network disarrangement (Westerberg et al., 2012), are hallmarks of psychiatric and neurodegenerative diseases. Clearly, brain diseases are associated with dysfunction of neural systems. Although the outstanding question persists of whether such dysfunction is cause, consequence, or epiphenomenon, the notion that Systems-oriented research will prove more fruitful than traditional approaches to discovering, and thus manipulating, the biological underpinnings of diseases, has already been voiced for autism (Rosenberg et al., 2015), and motivates therapeutic approaches such as deep brain stimulation in Parkinson's disease (Ashkan et al., 2017). We anticipate that optogenetic and chemogenetic stimulations will be the most productive avenues in the emerging field of Clinical Systems Neuroscience (K. V. Kastanenka, Herlitze, S., Boyden, E.S., Tsai, L-H and Bacskai, B.J., 2017). *First*, these approaches offer the advantage of selective actions at the network and cellular levels—critically allowing the assessment of neuronal *versus* astrocytic effects—since viral vectors may be targeted at specific regions

through stereotaxic surgery. *Second,* they enable preclinical research in rodents and primates to demonstrate *causality* between network dysfunction and disease hallmarks (K. V. Kastanenka et al., 2017). *Third,* advances in viral vector technology for gene transfer significantly reduce vector-associated cytotoxicity and immune responses (Lundstrom, 2018), rendering chemogenetics and optogenetics amenable for clinical use in human patients.

8. Concluding remarks

We started this perspective article by posing several questions to guide the analysis of the role of astrocytes within Systems Neurosciences. We looked for initial answers in available studies that include measurements of astrocyte Ca^{2+} activity, targeted optogenetic and chemogenetic manipulations, and complex behaviors or neural networks. We asked whether astrocytes are as functionally heterogeneous as neurons. We contend that they are not. We put forth anatomical, molecular, and computational arguments in support that astrocytes may operate modules akin to mini-circuits in large scale networks, performing canonical computations throughout the brain. Mathematical analyses of *in vivo* data together with *in silico* modeling will be necessary to firmly establish the existence, and nature, of astrocytic computations, and whether they encode specific variables. We may get closer to the answer using decoding approaches in reward-associated decision making over variable contexts, a complex behavioral paradigm in which the brain needs to perform difficult computations within the slow time scale of astrocytic Ca^{2+} signals. Another question was whether astrocytes use Ca^{2+} to carry out spatiotemporal integration of multicellular signals. A first insight is that there is behavior-dependent integration in a time scale of sub-seconds to supra-seconds, perhaps driven by signal thresholding and timing control. We propose to use dimensionality reduction, a tool developed in the context of machine learning, to identify the minimum amount of ROIs that carry independent information in Ca^{2+} transients in different contexts. This is a mandatory step towards finding structure in these transients, with the assumption that astrocytic Ca^{2+} responses behave like a dynamical system that can adopt multiple regimes. Thus, the question of whether subcellular compartments in astrocytes perform different functions ought to be reformulated to whether there are function-specific Ca^{2+} regimes. Further, we identify technical and analytical shortages in joint astrocyte- and neuron-population imaging, and ensuing data processing algorithms. Finally, we point to theoretical frameworks used by Systems Neurosciences that might benefit from the inclusion of astrocytes. Many avenues of exploration remain. To cite two, we have the role of astrocyte-based computations in long-term processes underlying memory, perhaps by intervening in memory replay in the so-called resting brain, and the failure of neural circuits including astrocytes in neurodegenerative and psychiatric diseases. Decoding astrocytes may represent a leap forward towards novel approaches in the study of astrocytes in health and disease.

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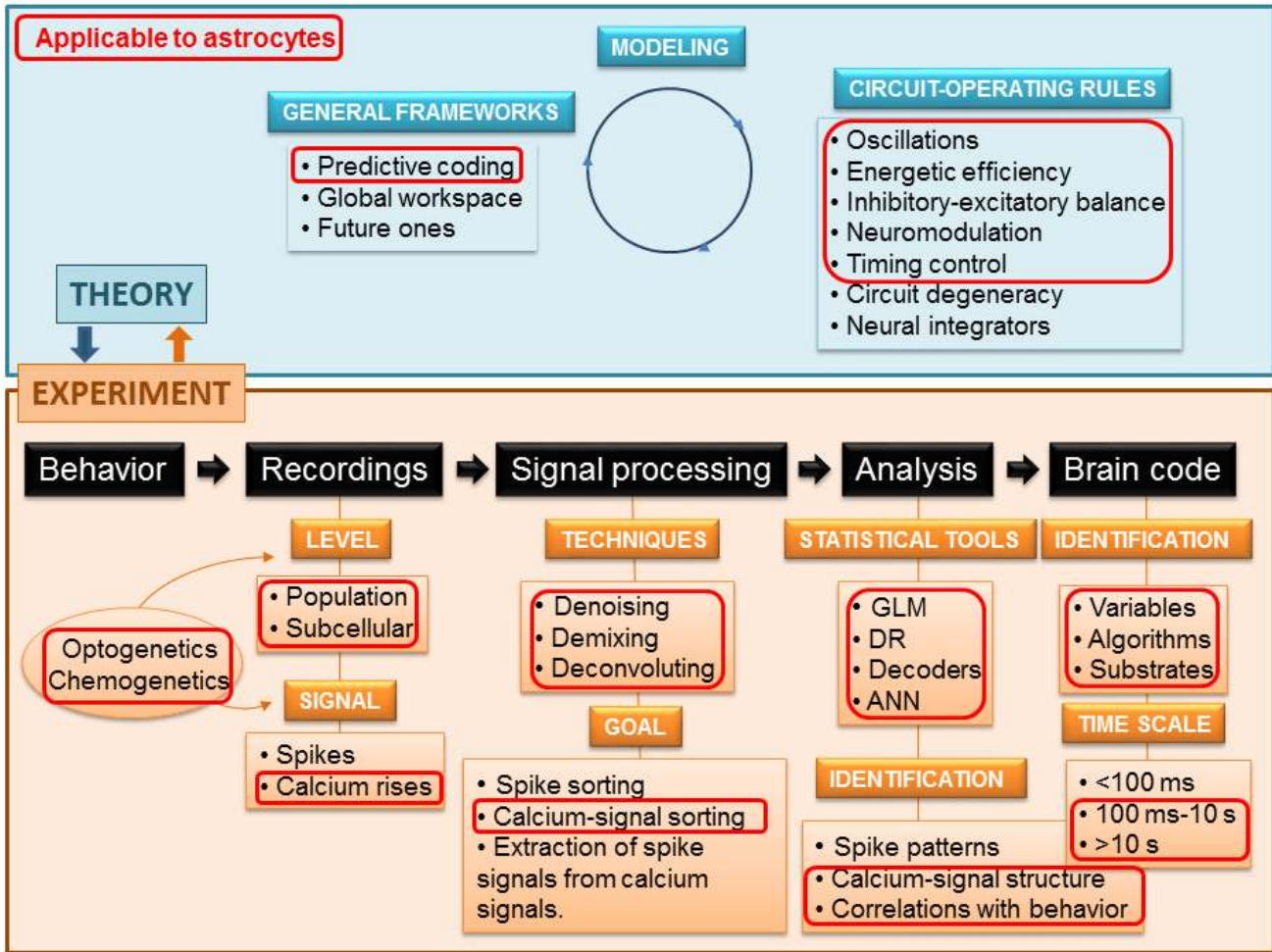
Figure 1. Workflow in Systems Neuroscience. A central problem in Neuroscience is to explain how electrical and chemical signals are used in the brain to represent and process information. The workflow depicts the stages and tools currently used to decipher neuronal codes. In red squares we highlight the elements that are relevant to the study the role of astrocytic Ca^{2+} in neuronal coding.

Table 1. System-like studies in astrocytes					
Direction of experimental manipulation	Stimulation	Neural circuits	Readouts	References	Predicted canonical computations
BEHAVIOR ↓ ASTROCYTES	Sensory stimulation	Barrel cortex	Astrocytic Ca²⁺; LFP; local postsynaptic activity	(X. Wang et al., 2006)	• Filtering • Thresholding • State switching
			Astrocytic Ca²⁺; LFP; brain state	(Takata et al., 2011)*	
		Visual cortex	Astrocytic Ca²⁺; neuronal Ca²⁺; hemodynamic responses	(Schummers et al., 2008) (Stobart et al., 2018)	
			Astrocytic Ca²⁺; EPSP; IPSP; SIC; patch-clamp recordings; visual response selectivity	(Perea et al., 2016)*	• Gain control
		Hippocampus	Astrocytic Ca²⁺; LTP; CA1 post-synaptic depolarization	(Navarrete et al., 2012)	• Thresholding • Coincidence detection • Gain control
	Neuromodulation	Cholinergic	Astrocytic Ca²⁺; LFP; brain state	(Takata et al., 2011)*	• Thresholding • Coincidence detection • Gain control • E/I balance
		Noradrenergic	Astrocytic Ca²⁺; EcoG	(Ding et al., 2013)	
			Astrocytic Ca²⁺; locomotion; electromyography	(Paukert et al., 2014)	
ASTROCYTES ↓ BEHAVIOR	Optogenetics	Cerebellum	Glutamate release; EPSP; LTD; motor behavior	(Sasaki et al., 2012)	• Gain control
		Somatosensory cortex	Astrocytic Ca²⁺; neuronal Ca²⁺; LFP; glutamate release; brain state	(Poskanzer & Yuste, 2016)	• Gain control • E/I balance • State switching
		Visual cortex	Astrocytic Ca²⁺; EPSP; IPSP; SIC; patch-clamp recordings; visual response selectivity	(Perea et al., 2016)*	• Gain control
		Brain stem	Astrocytic Ca²⁺; ATP release; neuronal membrane potentials; EPSC; breathing	(Gourine et al., 2010)	• Gain control • Gain control • E/I balance
		Hypothalamus	Sleep	(Pelluru et al., 2016)	
			Adenosine release; open-field behavior; food intake	(Sweeney et al., 2016)	
			Astrocytic Ca²⁺; patch clamp recordings; IPSC; food intake	(Chen et al., 2016; L. Yang et al., 2015)	
	Chemogenetics	Hippocampus	Astrocytic Ca²⁺; LTP; EPSC; memory acquisition; contextual and spatial memory	(Adamsky et al., 2018)	
		Amygdala	Astrocytic Ca²⁺; IPSC; EPSC; fear-expression	(Martin-Fernandez et al., 2017)	• Gain control • E/I balance
LFP, Local field potentials, LTD, long-term depression, LTP, long-term potentiation, EPSP, excitatory postsynaptic potential, IPSP, inhibitory postsynaptic potential, ECoG, electrocorticogram recordings, SIC, slow inward currents, *Belonging to more than one category					

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