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Chapter 12

Noradrenaline Drives Structural Changes in Astrocytes and Brain Extracellular Space

Ang D. Sherpa[1,2,✉], Chiye Aoki[2,✉] and Sabina Hrabetova[1,✉]
[1]State University of New York Downstate Medical Center, Brooklyn, NY, United States, [2]New York University, New York, NY, United States

Chapter Outline

ABSTRACT
Locus coeruleus neurons innervate multiple brain regions. These neurons release noradrenaline through their axonal varicosities into the extracellular space through synaptic and volume transmission during states of arousal. The extracellular space is a channel that surrounds brain cells, facilitating diffusion-mediated transport of signaling molecules, ions, and drugs. Distal astrocytic processes expressing β-adrenergic receptors are targets of noradrenaline. In this review, we discuss work in cortical tissue indicating that β-adrenergic agonist, isoproterenol, expands astrocytic processes. Isoproterenol-driven increase in volume of astrocytic processes contributes partially to decrease in the extracellular space volume from 22% to 18%. Decrease in the extracellular space volume suggests increased concentration of ions, neurotransmitters, and neuromodulators diffusing in the

✉Correspondence address
E-mail: ads420@nyu.edu; ca3@nyu.edu; sabina.hrabetova@downstate.edu

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extracellular space, which, in turn, facilitates neuronal signaling during noradrenaline release in cortex.

Keywords: Noradrenergic system; volume transmission; astrocytes; diffusion; extracellular space; volume fraction; tortuosity; real-time iontophoretic (RTI) method; electron microscopy; sleep–wake cycle

THE NORADRENERGIC SYSTEM—GENERAL REMARKS

Noradrenergic signaling is implicated in learning and memory, attention, anxiety and stress, arousal, and mood[1,2] and in experience-dependent cortical plasticity.[3–6] A major source of noradrenergic signal in the central nervous system arises from a collection of brain stem neurons, called locus coeruleus (LC). LC neurons synchronously fire together in tonic and phasic modes. A single LC neuron innervates various brain regions, including the cortex, the cerebellum, the hypothalamus, and the spinal cord, through axonal branching to exert a widespread synchronous influence on these brain circuits.[7] Recent work also suggests that there are functionally and anatomically separate LC projection groups, since Chandler et al.[8] showed that LC neurons innervating subregions of the prefrontal cortex are distinct from those innervating the motor cortex.

DIVERSITY OF NORADRENERGIC RECEPTOR EXPRESSION UNDERLIES DIVERSITY OF ASTROCYTIC RESPONSES

The functional outcome of noradrenaline (NA) released from axonal varicosities of LC neurons depends on the distribution of noradrenergic receptors among brain cells within and across brain regions. All three noradrenergic receptors (ARs), α_1 (subtypes: α_{1A}, α_{1B}, and α_{1D}), α_2 (subtypes: α_{2A}, α_{2B}, α_{2C}, and α_{2D}), and β (β_1, β_2, and β_3), are metabotropic G-protein coupled receptors. In neurons, α_1-AR and β-AR are postsynaptic while α_2-ARs are both pre and postsynaptic and β_2-ARs are shown to act presynaptically.[1] In astrocytes, activation of α_1-ARs activates the enzyme, phospholipase C, that catalyzes the conversion of phosphatidylinositol 4,5-bisphosphate into two signaling molecules, inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol. The released IP_3 binds to a transmembrane glycoprotein on the endoplasmic reticulum called IP_3 receptor ($InsP_3R$), which induces a conformational change in $InsP_3R$ that subsequently allows $InsP_3R$ to release calcium from the endoplasmic reticulum into the cytoplasm.[9] Aoki et al.[10] localized α_{2A}-ARs on postsynaptic membranes and in nonsynaptic locations of axons, dendritic shafts, and astrocytic processes in the monkey dorsolateral prefrontal cortex. Astrocytic α_2-ARs interact preferentially with G_i-proteins that inhibit adenylyl cyclases and 3′,5′-cyclic adenosine monophosphate (cAMP) production. Within astrocytes, inactivation of adenylyl cyclases promotes

glycogenesis, but through its βγ subunit, α_2-ARs also couple positively with protein kinase C and calcium to promote glycogenolysis under certain situations.[9]

Activation of β-ARs stimulates adenylyl cyclases, which, in turn, increase the formation of cAMP. Upregulation of cAMP within astrocytes activates cAMP-dependent protein kinases that phosphorylate cytoskeletal proteins, such as glial fibrillary acidic protein (GFAP) and vimentin to alter the morphology of astrocytic processes[11,12] and glycogen phosphorylase to promote glycogenolysis.[9] Through these downstream effectors, adrenergic receptors (α_1, α_2, and β) contribute to the many astrocytic functions such as glycogen metabolism, immune response regulation, release of neurotrophins, such as brain-derived neurotrophic factor and nerve growth factor, and morphological changes.[9,13,14] Astrocytic β-ARs are also implicated in brain diseases, such as Alzheimer's disease, stroke, multiple sclerosis, and human immunodeficiency virus encephalitis.[13]

Using immuno-electron microscopy, Aoki[15] found a ninefold greater encounter of β-ARs on astrocytic processes of adult cortex, compared to neuronal processes, suggesting that nonneuronal component is more responsive to NA. This notion is supported by a recent study showing a robust calcium signaling induced in neocortical astrocytes but not neurons by NA, acting predominantly through α_1-ARs.[16] Additionally, it is important to note that β-ARs expressed on astrocytes that ensheath synapses occur displaced from noradrenergic terminals.[15,17] The distribution of ARs on multiple cell targets such as neurons, glia, and microglia within the central nervous system[18,19] suggests that NA released into the surrounding extracellular space (ECS) can have multiple targets besides the synaptic junction. This is achieved through volume transmission, i.e., extrasynaptic diffusion-mediated transport of transmitters, modulators, trophic factors, and neurotransmitters, including NA, in the ECS,[14] that allows for interactions among their many targets.

NORADRENERGIC SYSTEM RELATES TO FUNCTION OF ASTROCYTES

Astrocytes are a major type of glial cells in the central nervous system and provide many additional functions. Astrocytes participate in maintaining ionic composition and pH of extracellular fluid,[20] and they accommodate a powerful uptake system for a major excitatory neurotransmitter, glutamate.[21] Astrocytes express the major Na^+-dependent glutamate transporters GLT-1 (Glutamate Transporter-1) and GLAST (Glutamate Aspartate Transporter) that remove extracellular glutamate released during synaptic transmission, thereby preventing excitotoxicity.[21] Once accumulated inside astrocytes, glutamate is converted to glutamine with the enzyme glutamine synthetase and is transported back to neurons.[22] Application of an α_1-AR agonist, phenylephrine,

increases glutamate uptake in vivo, indicating that the noradrenergic signal intensifies glutamate uptake.[23] Astrocytes are involved in maintaining extracellular level of potassium ($[K^+]_{ECS}$) around 3 mM. During neuronal activity, $[K^+]_{ECS}$ increases to 10–12 mM, and during anoxic depolarization associated with ischemic conditions in brain, $[K^+]_{ECS}$ can rise to become as high as 70 mM.[24,25] Astrocytes remove excess potassium through passive "spatial buffering," facilitated by inwardly rectifying K^+ channels and coupling of astrocytes via gap junctions, and through a Na^+/K^+-ATPase pump that pumps K^+, along with water molecules, inward of astrocytes.[26,27] Astrocytic β-AR agonist, isoproterenol (ISO), activates Na^+/K^+-ATPase, to increase the removal of $[K^+]_{ECS}$.[28] Thus, the noradrenergic system enables astrocytic functions through α_1-AR and β-ARs. Astrocytes also express water channels, aquaporin 4 (AQP4) that contribute to water homeostasis and transport within brain.[29,30] While functional interplay between the AQP4 channels and the NA system remains to be tested, it has been shown that the levels of NA are increased in the medial prefrontal cortex of AQP4-knockout mice.[31] In short, through a variety of mechanisms, astrocytes modulate neuronal communication and make an impact on excitability of neuronal population, both of which may be enhanced further by the release of a number of substances from glia.

Morphology of astrocytes and their distribution in the neuropil are well suited in accomplishing these diverse functions. A small cell body of an astrocyte is surrounded by a network of astrocytic processes. Proximal astrocytic processes extending from the cell body are less numerous but thick and contain the intermediate protein, GFAP,[32,33] which undergoes phosphorylation in response to changes in cAMP level that, in turn, alter astrocyte morphology.[11,12] The proximal astrocytic processes extend into morphologically complex distal processes devoid of GFAP. In contrast to the proximal processes, distal astrocytic processes are very thin and thread-like but account for about 85% of an astrocyte's volume.[34] These fine distal astrocytic processes are interposed between neurons and their processes, often wrapping structures, such as synapses. They are also positioned at the interface between the brain and surrounding tissue, forming glia limitans that line the pia mater or the astrocytic endfeet on blood vessels.[32,35] In fact, since these distal fine processes express ARs, transporters, and channels, they are the main functional domain of astrocytes. As will be described below,[36] we have evidence indicating that these fine distal processes that are devoid of GFAP nevertheless undergo morphological changes induced by β-ARs. These changes may occur via depolymerization of F-actin,[37] which cannot be visualized readily by conventional electron microscopic methods.

Astrocytic processes need to be distributed throughout the neuropil in order to be effective in ionic and water regulation and, indeed, they are.

At the same time, astrocytic processes need to be present at specific locations within the neuropil (e.g., close to synapses) in order to remove excess glutamate from perisynaptic regions as well as to participate in the modulation of synaptic transmission. This is accomplished through a uniform distribution of astrocytes within the neuropil. It has been reported that each astrocyte occupies a separate anatomical domain, resulting in a nonoverlapping tiled layout in the neuropil.[34,38,39] Because of their complex morphology and uniform distribution, astrocytes function as diffusion barriers for signaling molecules, including neuromodulators and ions released into the ECS synaptically and extrasynaptically, thereby contributing to the structural and molecular properties of the ECS.

NORADRENERGIC SYSTEM'S EFFECTS ON ASTROCYTES IN VITRO

Noradrenergic signal can induce morphological reorganization of astrocytic processes and morphological changes of astrocytes in culture and hypothalamic slices, where ISO has been used to activate β-AR on cultured astrocytes.[11,40] Cultured astrocytes from adult rat neurohypophyses[41] and astrocytes cultured from rat cortex at postnatal days 2–3[12] alter their morphology upon activation of β-AR. Bicknell et al.[40] found that pituicytes cultured from neurohypophyses are transformed from amorphous to stellate morphology when a β-AR agonist, ISO, is added to the medium. Vardjan et al.[12] performed quantitative analysis of astrocytes cultured from cortical brain tissue during incubation with ISO. It was reported that the cross-sectional area of astrocytes is reduced while the perimeter of astrocytes is increased upon β-AR activation, and that cultured astrocytes acquire a stellate morphology. We note that Vardjan's morphometric analyses focused on the more proximal astrocytic processes containing GFAP detected by immunolabeling, and therefore the fine GFAP-negative portions of astrocytic processes that could not be detected by confocal microscopy were excluded from this analysis.

NORADRENERGIC SYSTEM'S EFFECTS ON ASTROCYTES IN SITU

Aoki[15,42] reported a rich expression of β-AR at neuronal membranes of cortical synapses neonatally, but of their dominance at astrocytes in adulthood. Based on this study, Sherpa et al.[36] speculated that noradrenergic input to the adult visual cortex is likely to change the morphology of astrocytic processes, and that this morphological change, in turn, changes the structure of ECS and impacts the diffusional transport of substances in the ECS. In contrast to previous studies in cultured astrocytes, the β-AR agonist, ISO, was applied to astrocytes embedded within the microenvironment of intact neuropil of acutely prepared adult visual

cortex slices, and the analysis was focused on fine, distal astrocytic processes, previously estimated to occupy 85% of an astrocytic volume.[34] In agreement with previous studies in culture,[12] Sherpa et al.[36] reported significant changes of astrocytic morphology.

Sherpa et al.[36] found that ISO induced changes in several parameters of astrocytic morphology using electron microscopy (Fig. 12.1). First, the

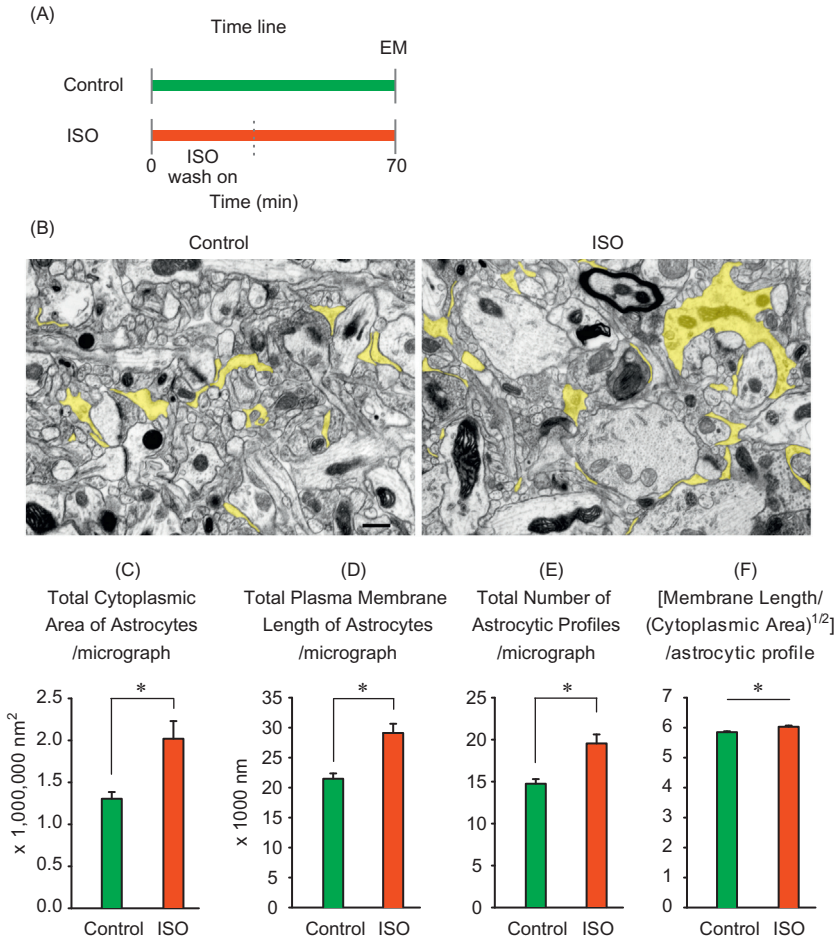

FIGURE 12.1 **Quantitative analysis of the morphology of astrocytic processes.** (A) Time line of electron microscopy experiment. (B) Representative electron microscopic images of neuropil of the visual cortex under control and ISO conditions. Astrocytic profiles are colored in yellow. Scale bar: 500 nm. (C–F) Summary of morphometric analyses (mean ± SEM). Control, green; ISO, red; asterisk, significant. *Reproduced from Sherpa AD, Xiao F, Joseph N, Aoki C, Hrabetova S. Activation of β-adrenergic receptors in rat visual cortex expands astrocytic processes and reduces extracellular space volume.* Synapse. *2016;70:307–316, with the permission of John Wiley & Sons, Inc.*

total cytoplasmic area of astrocytic processes was increased, suggesting expansion of distal astrocytic processes. Second, the total plasma membrane length of astrocytic processes encountered within electron micrographs was increased, suggesting new membrane synthesis. Third, the total number of astrocytic profiles encountered per unit area was increased. Fourth, the ratio of the plasma membrane length to (cytoplasmic area)$^{1/2}$ for each astrocytic profile was increased. Changes in the last two parameters suggest the formation of additional astrocytic profiles after ISO treatment.

All findings of Sherpa et al.,[36] except for the first one, agree with quantitative analyses of cultured astrocytes exposed to ISO.[12] While Sherpa et al.[36] found an increase in the total cytoplasmic area of astrocytic processes, Vardjan et al.[12] reported that the cross-sectional area of astrocytes was reduced upon β-AR activation. As alluded to above, this discrepancy may arise from the different domains of astrocytes that were studied: while Sherpa et al.[36] analyzed distal astrocytic processes, Vardjan et al.[12] analyzed the astrocytic cell body and proximal processes. It is likely that these results are, in fact, in agreement with each other. As a cultured astrocyte changes from the amorphous to stellate morphology, its cytoplasm is likely to be redistributed between the cell body and the distal processes. This would result in findings reported by Vardjan et al.[12] and Sherpa et al.[36] Taken together, study of astrocytic morphology in intact brain neuropil suggests that β-AR-induced changes in astrocytic morphology may have important functional implications by allowing for repositioning of targets of signaling molecules, such as neurotransmitter transporters, ion channels, receptors (including β-AR), and aquaporin channels within brain neuropil.

Besides β-AR, astrocytes respond with activation of α_1-ARs during the release of NA in the cortex. Ding et al.[43] observed a widespread increase in astrocytic calcium level in cortical astrocytes of awake mice from activation of α_1-ARs. Later, Paukert et al.[44] observed that activation of astrocytic α_1-AR induces a rise of intracellular calcium level simultaneously in cerebellum and visual cortex during locomotion. The NA mediated enhancement in calcium signaling in astrocytes is thought to facilitate astrocytes in detecting changes in neural activity. β-AR-mediated morphological changes in astrocytes also occur over an area of visual cortical neuropil[36] similar to the increase in cortical calcium signaling during α_1-AR activation.

BRAIN EXTRACELLULAR SPACE

The β-AR-induced expansion of astrocytic processes contributes to the diffusion properties of brain ECS. Brain ECS is a large compartment of brain tissue formed by numerous narrow spaces that surround brain

cells. It occupies about 20% of the total brain tissue volume,[45,46] but the individual intercellular gaps are only about 30–60 nm wide.[47,48] The ECS is filled with ionic solution and macromolecules of the extracellular matrix (ECM), predominantly proteoglycans and glycosaminoglycans. The ECS has a fundamental role in brain function. It facilitates diffusional transport of neuroactive substances, nutrients, metabolites, and therapeutic agents, while also serving as a reservoir of ions and growth factors sequestered by the ECM. Extracellular concentration and distribution of these substances is determined by the ECS structure.

Early studies of the ECS structure employed electron microscopy, which has a power to resolve individual narrow intercellular channels. Today, the morphometric approaches are sparingly used whenever the ECS parameters need to be precisely quantified. The main drawback of morphometric approaches is that the conventional fixation procedures cause significant water redistribution, leading to distortion of the ECS structure.[47,49] It has been shown that aldehydes required for ultrastructural preservation causes greater than 10% loss of the ECS.[50]

Transport of substances in the ECS is primarily mediated by diffusion. Diffusion, in turn, can be exploited as an experimental tool to quantify macroscopic parameters of ECS structure in live tissue.[51] Diffusion-based methods that study the ECS employ small extracellular probe molecules, such as a cation, tetramethylammonium (TMA^+, MW 74), to quantify two parameters of the ECS structure: volume fraction and tortuosity. This method is called the real-time iontophoretic (RTI) method.[45] Since TMA^+ has been used mostly, it is also known as the TMA^+ method. The volume fraction (α) represents the proportion of tissue volume occupied by the ECS. The tortuosity (λ) quantifies the hindrance imposed on the diffusion process by the tissue, relative to an obstacle-free medium. Tortuosity is defined as $(D/D^*)^{1/2}$, where D is the free diffusion coefficient in a free medium and D^* is the effective diffusion coefficients in brain.[45,51] Alternatively, diffusion hindrance can be defined as diffusion permeability (θ), which is a ratio of the effective diffusion coefficient in the brain tissue and the free diffusion coefficient.[52] In isotropic healthy brain, α is about 0.2 and λ is about 1.6 (i.e., diffusion of a small extracellular probe molecule in brain tissue is slowed down about 2.5 times, relative to an obstacle-free medium).[46,51]

Light microscopic images of brain tissue may lead us to believe that brain microstructure is static. But that is far from the truth. Neuronal spines have been shown to be pruned during development and to grow or move during synapse formation and to underlie synaptic plasticity.[53] Similarly, astrocytic processes were shown to be highly mobile, and be attracted to sites of high neuronal activity.[54] Cellular elements thus appear to be in a state of constant change and rearrangement, and these processes contribute to brain plasticity essential for learning, adaptation,

and survival. Since the ECS compartment is a counterpart of the cellular compartments, its structure also changes. These changes may be acute and reversible, such as during physiological conditions, when neurons are activated or osmotically challenged.[55,56] These changes may also be permanent, such as during brain trauma and in disease states. For example, the ECS structure changes significantly in many pathological states associated with cellular edema.[55,57,58] These dynamic or permanent changes in the ECS structure impact the extracellular concentration and distribution of substances diffusing in the ECS.

NORADRENERGIC SYSTEM'S EFFECTS ON EXTRACELLULAR SPACE STRUCTURE

A number of RTI studies performed in anesthetized animals, mostly rodents, isolated brain slabs such as an isolated turtle cerebellum and acute brain slices reported that the ECS occupies about 20% of the total brain volume.[56] Until recently, it was assumed that this value of ECS volume would apply to awake brain state as well. However, a recent study from Maiken Nedergaard's group[59] reported that the ECS volume dramatically decreases during an awake state. In this study, RTI measurements were carried out in the cortex of mice during sleep, awake, and under anesthesia. The ECS volume recorded in sleeping animals at midday and in animals anesthetized with a ketamine–xylazine mixture ($\alpha = 0.23$) was in good agreement with previously reported values of the ECS volume within acute brain slices.[56] However, the value of α was only about 0.14, when animals woke up in the evening. This represents a significant decrease (40%) in α during the awake state. Interestingly, no significant change in tortuosity was found between the sleep state and the awake state. Xie et al.[59] also reported that noradrenergic signaling is involved in the ECS volume changes during the sleep–wake cycle. They showed that a cocktail of adrenergic antagonists applied on the cortical surface of an awake animal increased the ECS volume from 0.14 to 0.23, indicating that blockade of adrenergic activity reverses increase in the ECS volume from that of the awake to the sleep state.

The study by Xie et al.[59] raised several questions. First, which brain compartment alters its volume in a manner reciprocal to the ECS volume changes? Second, which adrenergic receptors are responsible for the ECS volume changes? The study by Sherpa et al.[36] provided some answers to these questions (Fig. 12.2). In this study, ultrastructural analysis with electron microscopy and diffusion analysis of the ECS parameters was carried out in acutely prepared slices of the rat visual cortex with and without exposure to the βAR agonist, ISO. As already described, ultrastructural analysis found that astrocytic processes expanded during ISO application. It was also reported that α significantly decreased from

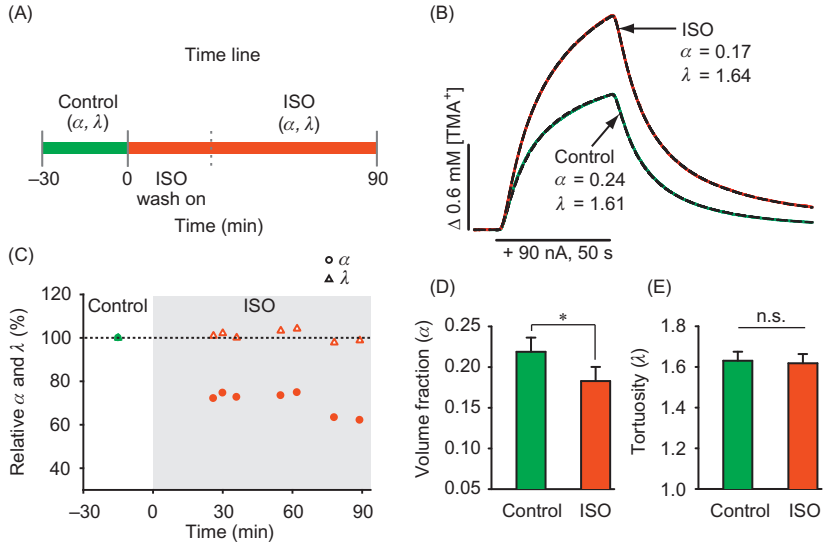

FIGURE 12.2 **Quantitative analysis of ECS parameters.** (A) Time line of RTI experiment. (B) Representative diffusion curves in the visual cortex under control (green) and ISO (red) conditions. Theoretical curves (dashed black) from the fitting procedure are superimposed. (C) Time course of α and λ values from one experiment. (D–E) Summary of α and λ values (mean ± SD). Asterisk, significant; n.s., nonsignificant. *Reproduced from Sherpa AD, Xiao F, Joseph N, Aoki C, Hrabetova S. Activation of β-adrenergic receptors in rat visual cortex expands astrocytic processes and reduces extracellular space volume.* Synapse. *2016;70:307–316, with the permission of John Wiley & Sons, Inc.*

0.22 to 0.18 in the ISO condition, while λ remained constant. The ECS volume obtained during ISO application in slices[36] is larger than during awake state in vivo.[59] This discrepancy may result from an enhancement of NA signaling from an intact LC in an awake brain whereas only a selective enhancement of NA signaling occurs in slices through βAR activation. Taken together, Sherpa et al.[36] identified astrocytes as one compartment that changes its volume in response to noradrenergic activation. Furthermore, this study identified βARs as one type of noradrenergic receptors involved in this process.

ECS structural parameters determine the spatiotemporal distribution of neuroactive substances diffusing in the ECS. As α is reduced under ISO conditions, cells and cellular processes in such a neuropil experience higher concentrations of released ions, neurotransmitters, and neuromodulators, and this enhances their actions on target sites. Our simulation model of extracellular diffusion in control conditions and in ISO conditions predicts such an effect (Fig. 12.3). Simulations show that the concentration of diffusion molecules reaches higher maximum and persists at a higher level in the ISO condition than in the control condition.

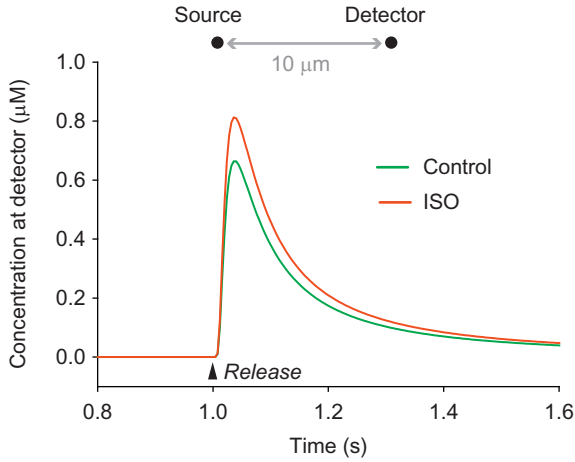

FIGURE 12.3 **Simulations of diffusion in the ECS.** In these simulations, informed by ECS parameters measured in the acute slices of the visual cortex in control and ISO conditions (Fig. 12.2), the same number of molecules was released (1 ms) from a point source and allowed to diffuse. Concentration profiles were recorded at a distance 10 μm from each source.

CONCLUSIONS

The noradrenergic system projects to the entire cortex and its functional outcome depends on the LC neurons innervating the cortex and diverse ARs situated among neurons, astrocytes, and microglia in the cortex. Recent work in the visual cortex reported that through volume transmission, NA activates β-ARs located on astrocytes, to expand distal astrocytic processes. Expansion of astrocytic processes accounts, partly, for the reduction in the ECS volume in cortex. Future studies may address whether this phenomenon is generalized to the other cortical regions that are innervated by the LC neurons. A reduced ECS volume will increase concentration of ions, neuromodulators, and neurotransmitters, leading to a signaling boost of these molecules on target sites. Owing to these changes in concentration of diffusing molecules in the ECS, we attribute a functional role to NA-driven expansion of astrocytic distal processes, i.e., modulation of communication among brain cells.

This review presents new important findings on how noradrenergic signal exerts its widespread effect on brain function through astrocytic morphology. It would be important in future to test for the β-AR-induced morphological changes in microglia, which also express β-ARs[19] and have received much attention regarding synaptic plasticity during the critical period for developmental plasticity.[60]

ABBREVIATIONS

AR adrenergic receptor
AQP4 aquaporin 4
cAMP 3′,5′-cyclic adenosine monophosphate
ECM extracellular matrix
ECS extracellular space
GFAP glial fibrillary acidic protein
GLAST glutamate aspartate transporter
GLT-1 glutamate transporter-1
IP_3 inositol 1,4,5-trisphosphate
ISO isoproterenol or isoprenaline
LC locus coeruleus
NA noradrenaline, norepinephrine
RTI real-time iontophoretic
TMA^+ tetramethylammonium

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