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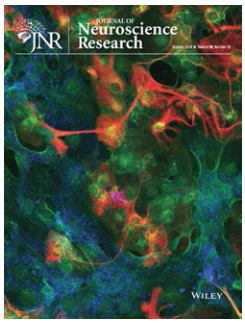
REVIEW



Locus Coeruleus and neurovascular unit: From its role in physiology to its potential role in Alzheimer's disease pathogenesis

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Abstract

Locus coeruleus (LC) is the main noradrenergic (NA) nucleus of the central nervous system. LC degenerates early during Alzheimer's disease (AD) and NA loss might concur to AD pathogenesis. Aside from neurons, LC terminals provide dense innervation of brain intraparenchymal arterioles/capillaries, and NA modulates astrocyte functions. The term neurovascular unit (NVU) defines the strict anatomical/functional interaction occurring between neurons, glial cells, and brain vessels. NVU plays a fundamental role in coupling the energy demand of activated brain regions with regional cerebral blood flow, it includes the blood–brain barrier (BBB), plays an active role in neuroinflammation, and participates also to the glymphatic system. NVU alteration is involved in AD pathophysiology through several mechanisms, mainly related to a relative oligoemia in activated brain regions and impairment of structural and functional BBB integrity, which contributes also to the intracerebral accumulation of insoluble amyloid. We review the existing data on the morphological features of LC-NA innervation of the NVU, as well as its contribution to neurovascular coupling and BBB proper functioning. After introducing the main experimental data linking LC with AD, which have repeatedly shown a key role of neuroinflammation and increased amyloid plaque formation, we discuss the potential mechanisms by which the loss of NVU modulation by LC might contribute to AD pathogenesis. Surprisingly, thus far not so many studies have tested directly these mechanisms in models of AD in which LC has been lesioned experimentally. Clarifying the interaction of LC with NVU in AD pathogenesis may disclose potential therapeutic targets for AD.

KEYWORDS

Alzheimer's disease, astrocytes, blood–brain barrier, locus coeruleus, neuroinflammation, neurovascular coupling, neurovascular unit, noradrenaline

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1 | INTRODUCTION

Alzheimer's disease (AD) represents the most frequent type of degenerative dementia worldwide (Scheltens et al., 2016). It is usually featured at the onset by the appearance of isolated episodic memory complaint (the so-called "mild cognitive impairment," MCI, due to AD), which later on is associated with multiple domain cognitive alterations and with the progressive need of support for daily life activities. The pathological features of AD are represented by intracellular accumulation of neurofibrillary tangles (NFTs) containing hyperphosphorylated Tau (p-Tau), and by extracellular accumulation of insoluble aggregates of β-amyloid (Aβ), also known as amyloid plaques (APs; Scheltens et al., 2016). In AD there is a progressive neuronal loss and frank atrophy at the level of cortical structures participating in cognitive processes, which parallels the accumulation of NFTs (Schellenberg & Montine, 2012). Such progressive NFT accumulation has been classified by an "NFT staging" by Braak and Braak (1991), according to which stage I defines the earliest occurrence of NFT deposits in the trans-entorhinal region of the entorhinal cortex, and later stages define the progressive extension of NFT pathology also to other limbic areas, up to NFT stage VI, in which NFT can be observed also in the neocortex (Braak & Braak, 1991).

The precise mechanisms through which APs and NFTs accumulate in sporadic AD are still a matter of intense study and debate (Long & Holtzman, 2019). Whatever they are, it is widely accepted that, before the occurrence of cognitive symptoms, there is a prolonged presymptomatic phase during which the pathogenetic mechanisms leading to AP and NFT formation develop (Dubois et al., 2014). In AD, different subcortical regions degenerate, and in particular, this is the case for the noradrenergic nucleus locus coeruleus (LC; as reviewed in Giorgi et al., 2017, 2019) and cholinergic nuclei of the basal forebrain (Hampel et al., 2018). LC, in particular, might start accumulating p-Tau decades before the onset of cognitive impairment (Braak, Thal, Ghebremedhin, & Del Tredici, 2011). An alteration of neurovascular coupling is considered as an early alteration occurring at cortical/subcortical level in AD, and brain fluorodeoxyglucose-PET and perfusion magnetic resonance imaging (MRI) studies showed the occurrence of relative oligoemia in those regions which maximally show pathological changes in AD before the onset of cortical neuronal loss in those same regions (Verclytte et al., 2016). Furthermore, cardiovascular risk factors are associated with AD (Scheltens et al., 2016), and in typical AD, APs and NFTs often coexist with subcortical microvessel alterations (Iadecola et al., 2019; Snyder et al., 2015), as well as with amyloid angiopathy (Greenberg et al., 2020), and this further confirms a role for altered neurovascular coupling in AD pathogenesis. In the last decades, several studies have detailed the so-called neurovascular unit (NVU) which defines the cellular elements participating in the fine-tuning of the physiological coupling between energy demand and cerebral blood flow (CBF; Iadecola, 2017); an early NVU alteration might be crucial for the pathogenesis of degenerative phenomena occurring in AD. The LC might significantly contribute to such an impairment as: (a) its fibers densely innervate the precapillary and capillary parts of NVU, (b) it is degenerated long before the occurrence of NFT/APs. Surprisingly, most of the literature addressing a role of LC on the proper functioning of the different components of NVU is sparse, dates back often to decades ago, and profited from a variety of experimental approaches which make sometimes very difficult to reconcile the results of different studies to one another. In the present paper, after describing in detail the structure and function of NVU, we review the available studies in which LC influence on NVU has been assessed, as well as data supporting the involvement of NVU in AD pathogenesis. Finally, we try to reconcile the available data from these research fields in a more comprehensive *scenario* in which LC degeneration may contribute significantly to AD pathogenesis by its effects on NVU.

Significance

Alzheimer's disease (AD) is featured by amyloid and neurofibrillary tangle deposits in the brain, but its pathogenesis has not yet been understood. The main brain noradrenergic nucleus, the locus coeruleus (LC), degenerates early in AD patients. LC, by innervating brain precapillary and capillary vessels, regulates neurovascular coupling and controls astrocytes, endothelial cells, pericytes, and the "glymphatic system," which together constitute the so-called neurovascular unit (NVU). As there is much evidence for an early NVU involvement in AD, we hypothesize that LC degeneration might play a significant role in this regard. We discuss the available experimental literature supporting such a view.

2 | THE NEUROVASCULAR UNIT

The NVU is the anatomical and functional complex that has been identified as crucial for the coupling of CBF with neuronal activity. The NVU is composed of neurons, glial cells (mainly astrocytes but also microglia), of cells contributing to the vessel wall, namely endothelial cells (EC), pericytes (PC), and smooth muscle cells (SMC), as well as of components of extracellular matrix (ECM) and endothelial *membrana basalis* (BM; Muoio, Persson, & Sendeski, 2014). NVU, aside from mediating the strict balance between the neuronal energy demand and the vascular supply, and maintaining the integrity of the blood–brain barrier (BBB), plays also a pivotal role in the clearance of waste substances and neuronal catabolic toxic by-products (Iadecola, 2017).

The close relationship between the neuronal and vascular compartments of the brain starts early during ontogenesis, when neural cells and mesenchymal cells, originating from different embryonic leaflets, strictly interact with one another in many brain development steps (Iadecola, 2017): on one hand, proliferating neural progenitors induce vascular arborization, guided by neurons' energy and

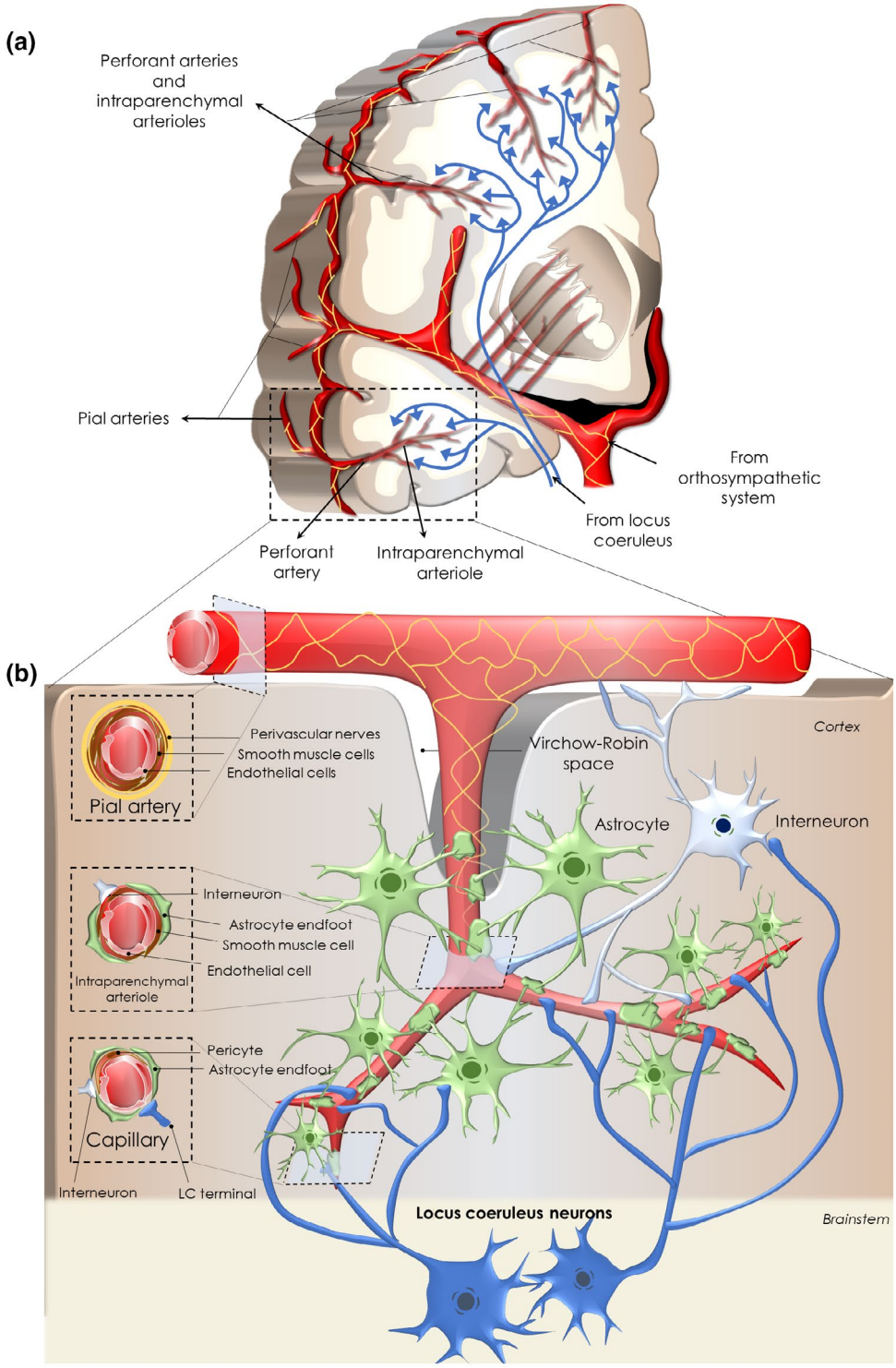

FIGURE 1 Noradrenergic innervation of the neurovascular unit (NVU). The figure represents schematically the main features of the noradrenergic (NA) innervation of the vascular tree (Panel A, upper part of the figure) and, more in detail, the basic features of the different components of the NVU with an emphasis on their innervation by the fibers originating from the pontine nucleus locus coeruleus (LC; panel B, lower). (a) The neurovascular tree receives both peripheric and central NA innervation. The former is provided by the orthosympathetic system, through post-ganglionic fibers originating from superior cervical ganglion; these autonomic NA projections reach extracranial cerebral vessels (carotid arteries, vertebral arteries, and jugular veins) and intracranial ones (pial arteries and veins and perforant arteries). The LC provides the so-called central NA innervation. Locus coeruleus noradrenergic fibers (LC-NA) innervate smaller size vessels, that is, intraparenchymal arterioles (IPA) and capillaries. (b) Orthosympathetic fibers travel along vascular walls, forming a nervous perivascular plexus that surrounds pial and perforant arteries. Instead, LC-NA fibers reach IPA and capillaries traveling within noradrenergic dorsal and lateral bundles, ending both with synaptic terminal and varicosities. At the capillary level, LC-NA terminals contact the NVU, targeting directly astrocytes end feet and pericytes, while endothelial cells and microglia are more likely to be modulated through volume transmission

oxygen demand (Coelho-Santos & Shih, 2020); on the other hand, newly developed vessels support neuronal polarization and migration of astrocytes and oligodendrocytes (da Silva, Campos, Gomes, & Stipursky, 2019).

The concept of NVU applies to the neuronal/vascular interface along the whole brain vascular system, from the subarachnoid arteries to the intraparenchymal capillaries. According to the aim of this paper, we will arbitrarily divide the neurovascular tree into two parts based on the source of noradrenergic innervation: the one under the control of the peripheric sympathetic system, mainly composed of larger arteries, and the one modulated by fibers originating from the nucleus LC (see below).

2.1 | The neurovascular tree

Arterial blood reaches the brain through the internal carotid and vertebral arteries. These merge at the level of the base of the forebrain, forming the circle of Willis; cerebral arteries originating from the circle of Willis run on the brain surface, and give rise to smaller branches, surrounded by pia mater, the so-called pial arteries. All of the abovementioned arteries are innervated by noradrenergic post-ganglionic orthosympathetic fibers originating from the superior cervical ganglion, and by cholinergic parasympathetic fibers provided by post-ganglionic neurons belonging to the sphenopalatine ganglion (Fornai & Lenzi, 2020; Iadecola, 2017; Nelson & Rennels, 1970) (Figure 1).

Pial arteries enter into the brain parenchyma, thus becoming "perforating arterioles" which are surrounded by an extension of the subarachnoid space, called perivascular space (PS) which at this level of the vascular tree is also often called "space of Virchow-Robin."

When further deepening into the brain parenchyma, perforating arterioles become smaller diameter "intraparenchymal arterioles" (IPA) which are precapillary meta-arterioles featured by a single vascular SMC layer surrounding the endothelium and BM, and by astrocyte end feet directly surrounding and getting in touch with SMC in the absence of clear PS. Terminal capillaries follow, in which SMC lack and are substituted by sparse PC placed within the BM. Thus, PS in these more distal branches is virtual and *de facto* substituted by the BM (Muoio et al., 2014). In line with this, astrocyte end feet surround and limit PS along penetrating arteries, while they directly contact the BM at the level of IPA and capillaries.

As said, the noradrenergic innervation of IPA and capillaries is no longer due to orthosympathetic fibers, but rather to fibers originating from LC which are in close connection with, and modulate all of the different components of the NVU, as detailed below (Cohen, Bonvento, Lacombe, & Hamel, 1996; Lecrux & Hamel, 2016; Figure 1). In this review we will focus mainly on this terminal part of NVU, indeed because of its LC-related innervation, and as it is considered the most important part of NVU regarding several functional aspects (Iadecola, 2017).

2.2 | The NVU cytoarchitecture

At the capillary level, the blood and the brain parenchyma are separated by the BBB which is formed by ECs tightly linked to one another by tight junctions (TJs) and adherens junctions (AJ; Abbott, Patabendige, Dolman, Yusof, & Begley, 2010). TJs are composed of two proteins, occludin and claudin, which cross the intercellular space; AJ are mainly constituted by cadherin, and represent the structural support for TJ organization (Greene & Campbell, 2016). TJ and AJ determine BBB impermeability.

The endothelium is surrounded by the BM, which is rich in collagen and proteoglycans and can be further divided into two layers: the vascular one, produced by ECs and PC, and the astrocytic one, produced by astrocytes (Xu, Nirwane, & Yao, 2018). BM integrity is fundamental for the correct functioning of BBB and, more in general, of the NVU; in fact, it provides structural and physical support for EC, PC, and astrocytes, which are anchored to the BM collagen through transmembrane glycoproteins, like integrins and dystroglycans (Thomsen, Routhe, & Moos, 2017). At the same time, the interaction between EC integrins and BM regulates the expression of endothelial TJ, thus modulating BBB permeability (Thomsen et al., 2017).

Pericytes, which are placed in the vascular layer of the BM, are star-shaped cells with a contractile ability and substitute SMC in the transition from arterioles to capillary (Brown et al., 2019). PC not only modulate the CBF by their contraction/relaxation (see Section 2.4), but they are also key in keeping local homeostasis and in regulating the permeability of BBB, being partly responsible also for its structural integrity (Sweeney, Ayyadurai, & Zlokovic, 2016). In particular, PCs promote production and assembling of BM proteins, like laminin and fibronectin; they also regulate TJ expression and modulate EC proliferation, communicating with the same EC through gap junctions and paracrine signals (Kolinko, Kralickova, & Tonar, 2018).

At precapillary/capillary level, NVU is encased by the end feet of astrocytes, which provide the functional and structural connection between neurons and vascular system (Abbott, Pizzo, Preston, Janigro, & Thorne, 2018; Marina et al., 2020). Astrocytes play a fundamental role in NVU function since they not only take part actively in neurovascular coupling (see Section 2.4), but they also induce and maintain BBB organization (De Luca, Colangelo, Virtuoso, Alberghina, & Papa, 2020); in fact, astrocytes release several inducing factors, like transforming growth factor-β, fibroblast growth factor (FGF), and angiopoietin 1, which stimulate ECs to form BBB (Abbott, Rönnbäck, & Hansson, 2006). At the same time, they regulate the water balance of interstitial space, mainly through the expression of the protein aquaporin-4 (AQP4), which abound in their end feet (Vandebroek & Yasui, 2020).

Finally, in the ECM surrounding capillaries there are also microglial cells and phagocytes which play mainly waste-clearing and immunological roles (Lee & Jayant, 2019; Presta et al., 2018).

2.3 | Relationship with the glymphatic system

The so-called glymphatic system (GlyS) is a relatively new concept, defining the functional structure which provides waste clearance and metabolites distribution via cerebrospinal fluid (CSF; Plog & Nedergaard, 2018). The CSF is produced by epithelial cells of the choroid plexus, and it flows through subarachnoid space, separated from cortical arteries by the pial membrane. CSF then follows perforant arterioles through PS, and then IPA, through the virtual space between the vascular wall and astrocytes end feet which form the so-called glial limiting membrane. At the level of brain capillaries this virtual space is eventually substituted by BM through which the CSF flows (Jessen, Munk, Lundgaard, & Nedergaard, 2015; Wardlaw et al., 2020). Astrocytic AQP4 allows the flow of water from the PS/BM to the IS (Vandebroek & Yasui, 2020), and this determines a bulk convection flow toward astrocyte end feet surrounding the perivenular space, which constitutes the efflux route of the GlyS (Plog & Nedergaard, 2018). The GlyS thus strongly participates in the clearance from the brain of waste substances accumulating into the IS (including β-amyloid; Iliff et al., 2012), as detailed in Section 6.4.

2.4 | Neurovascular coupling

The coupling of neuronal activity with blood/oxygen supply is likely to be regulated by two complementary mechanisms: a feedforward and a feedback one (Iadecola, 2017). In fact, neurons control a feedback circuit, releasing vasoactive substances that enhance the CBF in parallel with neural activity increase (Dalkara & Alarcon-Martinez, 2015). At the same time the vegetative nervous system (Brassard, Tymko, & Ainslie, 2017) and the nuclei belonging to the reticular formation (Cohen et al., 1996; Lecrux & Hamel, 2016) may act in a feedforward way, via "direct" effects on vessels. The net effect of these two mechanisms is CBF increase in the functionally activated cerebral region and its reduction in resting ones; this phenomenon forms the basis for BOLD (i.e., blood oxygen level-dependent) functional magnetic resonance imaging (fMRI) (Mathias, Plank, & David, 2017).

As said, neurons are considered the main driver of the neurovascular coupling (Muoio et al., 2014): when neuronal activity increases, glutamate released by synaptic terminals activates ionic glutamatergic receptors, namely *N*-methyl-D-aspartate and a-amino-3-hydroxy-5-methyl-4-isoxazol propionic acid receptors; this leads to increased intracellular concentration of Ca^{2+}, resulting in the activation of NO synthetase and esonucleotidase, which produce NO and adenosine, respectively, which are both strong vasodilator (Lecrux & Hamel, 2016). Besides, increasing evidence supports a fundamental role for GABAergic interneurons, which would be excited within active cortical regions, and may modulate the CBF by acting on astrocytes (Anenberg, Chan, Xie, LeDue, & Murphy, 2015) and also releasing co-transmitters such as neuropeptide-Y, parvalbumin (PV), somatostatin, or NO. At the same time, astrocytes are recruited also directly by neurons, via metabotropic glutamate receptor (mGluR) and ADP/ATP release: the rise of intracellular Ca^{2+} activates cyclooxygenase, and the synthesis of prostaglandin E2; it also increases extracellular K^+ release, leading to SMC relaxation (Marina et al., 2020). Endothelial cells, too, may contribute to neurovascular coupling, likely through K^+ currents which spread retrogradely through the endothelium, thus avoiding flow-stealing effects (Iadecola et al., 1993; Longden et al., 2017). Eventually, arteriolar SMC or capillary PC relaxation reduces vascular resistance, thus further increasing regional CBF (Smyth et al., 2018; Sweeney et al., 2016).

3 | NORADRENERGIC INNERVATION OF THE NVU

As already mentioned, the neurovascular tree is reached by NA projections originating from two different sources: the orthosympathetic nervous system and the pontine nucleus LC, which have been defined as "extrinsic" and "intrinsic innervation", respectively (Hamel, 2006; Figure 1).

3.1 | Peripheral or extrinsic noradrenergic innervation of the cerebral brain vessels

The "extrinsic noradrenergic innervation" is provided by post-ganglionic orthosympathetic fibers mainly originating from the superior cervical ganglion, which travel along bigger size brain arteries' wall surface and eventually innervate also pial arteries and first-order penetrating arterioles (Hamel, 2006). These nerve fibers constitute a dense network around larger arteries, while they mostly end as isolated fiber terminals on arterioles (Edvinsson, 1987). These vessels express α1 adrenergic receptors (AR) and β1/2-AR, which differently regulate vasoconstriction/vasodilation (Brassard et al., 2017; Fornai & Lenzi, 2020; Owman, Edvinsson, & Nielsen, 1974).

3.2 | Central or intrinsic noradrenergic innervation: The locus coeruleus

The "intrinsic noradrenergic innervation" of brain vessels is provided by the noradrenergic pontine nucleus LC. LC is the main noradrenergic nucleus of the central nervous system; it is a tube-shaped aggregate of noradrenergic neurons placed below the fourth ventricle floor, which sends efferents to the whole brain (Bucci et al., 2017; Fernandes, Regala, Correia, & Gonçalves-Ferreira, 2012; Nagai, Satoh, Imamoto, & Maeda, 1981). LC belongs to the so-called isodendritic core of the brainstem reticular formation, and it is characterized by receiving afferences from a variety of structures, and by sending diffuse projections throughout the whole brain (Berridge & Waterhouse, 2003; Theofilas, Dunlop, Heinsen, & Grinberg, 2015). LC efferent fibers reach cortical and subcortical regions *via* two main axon fiber pathways: a dorsal bundle, reaching the neocortex, hippocampal formation, and thalamus; and a lateral bundle, for the

amygdala and olfactory cortex (Szabadi, 2013). LC axons, aside from establishing synaptic contacts with target cells, can also release noradrenaline (NA) from varicosities which are distributed along the fibers themselves, thus producing the so-called "volume transmission" (Fuxe, Agnati, Marcoli, & Borroto-Escuela, 2015). In these ways, a single LC efferent fiber can target several types of postsynaptic cells simultaneously (Aston-Jones & Waterhouse, 2016). At the level of the NVU, LC axonal terminals get in contact with astrocytic end feet, BM, and EC (see below).

3.3 | Ultrastructural studies

There are only a very few morphological studies specifically addressing the interactions of NA terminals with different components of the NVU, and these have been obtained mainly in the neocortex of rodents.

They have shown that: (a) the NA terminals originating from the LC densely innervate the intraparenchymal vessels and the brain capillaries, (b) many of these terminals directly get in touch with astrocyte end feet, (c) some of the NA terminals directly innervate parts of the BM which are not covered by ECs.

The fact that axon terminals of fibers originating from LC neurons innervate IPA and capillaries, while pial arteries are innervated by NA terminals originating from the superior cervical ganglion, has been shown by ultrastructural studies, indeed. These have been performed in animals that underwent LC lesioning either by direct morphological observation (e.g., see Cohen, Molinatti, & Hamel, 1997) or indirectly (Kalaria, Stockmeier, & Harik, 1989), and in animals that underwent cervical ganglionectomy (e.g., Duverger et al., 1987). Actually, at which precise level of the parenchymal brain vessels tree there is a switch between orthosympathetic and LC-related NA innervation is not fully clear yet, that is, it has not been detailed whether the penetrating arterioles which follow pial ones, and which still show PS, already bear some terminals from LC. In any case, as already repeated above, it is widely accepted that smaller IPA (or metarterioles) and capillaries are exclusively contacted by fibers originating from the LC (Cohen et al., 1997).

As said, the abovementioned observations on NA terminals and microvascular interaction derive mainly from a few seminal ultrastructural studies (Cohen et al., 1997; Paspalas & Papadopoulos, 1996). They allowed even estimating that NA terminals cover approximately 60% of the surface of the capillary bed (Paspalas & Papadopoulos, 1996). In the vast majority, NA terminals interact with microvasculature indirectly, that is by contacting astrocyte end feet or PC: indeed, in their elegant study Cohen et al. clearly showed that up to 75% of the NA terminals closest to the vessel's wall (i.e., <0.25 μm aside) were in contact with a perivascular astrocyte (Cohen et al., 1997), but that, in any case, there was a significant contact between NA terminals also considering those within 3μm from the vessel wall. These data were in line with what previously observed by Paspalas and Papadopoulos qualitatively (Paspalas & Papadopoulos, 1996). The latter authors showed clearly that in brain capillaries, occasionally it can be observed also the occurrence of a direct interaction of NA terminals with parts of the BM which are not covered by astroglial end feet (Paspalas & Papadopoulos, 1996). It is worth mentioning that Cohen et al. emphasized that perivascular NA fibers seldom establish clear synaptic junctions with glial or neuropil components of the NVU, but they are rather represented by varicosities (Cohen et al., 1997). In line with this observation, it has been proposed that the LC terminals modulate NVU function mainly by volume transmission. This further emphasizes how important it is for an effective modulatory role of LC on NVU, the close anatomical relation by its terminals and its different components.

3.4 | Adrenergic receptors in the different components of the NVU

Astrocytes express a variety of AR which have been associated with specific molecular and biochemical effects. In brief, the expression of AR has been assessed in different experimental paradigms: most of the studies have been performed in cultured or freshly isolated astrocytes collected from different brainstem regions as well as from cortical areas, and *in vivo* experiments in different animal species; all of these data have been obtained mainly in rodents. A detailed review of those different paradigms is beyond the aims of the current review but can be found in an extensive, even though not recent, literature analysis by Hertz, Chen, Gibbs, Zang, and Peng (2004). Concerning the expression and function of astrocyte AR receptors, it should also be emphasized that most studies analyzed astrocytes belonging to a specific part of the brain (e.g., frontal cortex, or specific nuclei of the brainstem) and did not assess astrocytes located close to microvessels, but rather astrocytes in general. We cannot rule out that those astroglial cells which are directly in contact with the microvessels and directly participate in the NVU might show a preferential expression of specific AR subtypes.

In any case, in brief, it has been shown that astrocytes express all AR subtypes, that is, α1 and α2, and β1, β2, and β3 receptors. β-AR are the most densely expressed AR in astrocytes (with an estimated expression of 4,000–6,000 receptors per astroglial cell [Hertz et al., 2004]), and it has been shown that at least in the cortex they are more represented in glial cells than in neurons (Morin, Sapena, Zini, Onteniente, & Tillement, 1997). β2-AR seems to be the AR subtype more expressed in astrocytes (Hertz et al., 2004). Concerning α-AR, it has been proposed that α1 is not always expressed by astrocytes and, in any case, its density is much lower than that of β-AR (Shao & McCarthy, 1993). Among α-AR, α2a seems to be significantly expressed on the astrocyte membrane, and they may be even more represented on glia than on neuronal dendrites (Enkvist et al., 2002).

The activation of AR expressed on astrocytes induces a variety of effects; for a detailed description of them, especially concerning metabolic ones, see the comprehensive review by Hertz et al. (2004). In brief, α-AR is involved in glycolysis and α- and β-AR are involved in glycogenolysis and synthesis. β-AR are involved significantly in

glutamate and GABA uptake, as well as in modulating significantly GAP junction permeability between interconnected astrocytes (see Hertz et al., 2004).

In the last decades, several studies have also detailed the effects of astrocyte AR activation on the synthesis and secretion of growth factors and the production of inflammation-related proteins: both phenomena are likely to play a crucial role in the involvement of NA-NVU interaction in neurodegeneration, as further described also in Section 7.

In particular, concerning the role of NA on growth factor (GF) synthesis, dating back to the late 1980s, several studies have shown that glial AR stimulation induces the synthesis and release of FGF, brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF; Follesa & Mocchetti, 1993; Furukawa, Furukawa, Satoyoshi, & Hayashi, 1987; Juric, Miklic, & Carman-Krzan, 2006; Kajitani et al., 2012; Krzan, Wu, & Schwartz, 2001; Mocchetti et al., 1989). In astrocytes, it has been shown that among AR: (a) β1,2 AR and α1-AR are strongly involved in BDNF production (Jurič, Lončar, & Čarman-Kržan, 2008), (b) FGF-2 is induced by via α- and β-AR (Kajitani et al., 2012), (c) NGF synthesis is mainly induced by β-AR stimulation (Follesa & Mocchetti, 1993; Schwartz & Mishler, 1990).

AR have been demonstrated also at the level of PC and ECs of capillaries, and have potentially relevant functional roles, as NA terminals can get as close as less than 0.25 μm from the endothelium (Cohen et al., 1997), and sometimes directly contact the BM, without glial end feet interposition (Paspalas & Papadopoulos, 1996).

Surprisingly, only very few studies specifically addressed the expression of AR in the endothelium of brain capillaries and showed that cultured EC express functioning α1/α2- and β1/β2-AR (Bacic, McCarron, Uematsu, & Spatz, 1992; Durieu-Trautmann, Foignant, Strosberg, & Couraud, 1991).

Finally, concerning brain capillary PC, Elfont, Sundaresan, and Sladek (1989) showed that these cells express α2- and β-AR, by profiting of radioligand-binding studies on bovine PC cultures derived from cerebral microvessels; β-AR expression on PC has also been confirmed more recently (Asashima, Iizasa, Terasaki, & Nakashima, 2003).

4 | EFFECTS OF NORADRENALINE RELEASED BY LC ON THE NVU

4.1 | Effects of LC activity on BBB

In the last decades, several studies have assessed the effects of LC lesion or stimulation, on either the BBB permeability/integrity or on CBF. Especially in the older studies, also the effects of NA or AR agonists/antagonists administration had been assessed; however, these are the ones which gave more conflicting results, and this is likely to be due to the variety of indirect effects exerted by these compounds on the LC discharge itself (Table 1). To further complicate the interpretation of data on LC, several studies assessed the effects of manipulation of NA system during pathological or stressful conditions (Table 1).

In the following paragraphs, we will briefly describe the main findings of these studies and we will try to define an overall *scenario* summarizing all of them. Table 1 reports details on these studies in terms of design and main results.

In the first study addressing these aspects, Raichle, Hartman, Eichling, and Sharpe (1975) showed that LC stimulation by carbachol microinjection induced a dramatic reduction of CBF and increased permeability of BBB to water in Rhesus monkeys which had been previously undergone superior cervical ganglionectomy to remove the contribution of sympathetic NA (Raichle et al., 1975). In rats, LC electrical stimulation has been shown to induce an increased permeability of the BBB to mannitol (Pavlasek, Haburcak, Haburcakova, Orlický, & Mikulajová, 1998) or, in a stimulation frequency-dependent manner, to fluorescein (Sarmento, Borges, & Lima, 1994).

The opposite, too, that is, the effect of LC lesion, has been assessed by several studies. In particular, LC has been lesioned by direct microinjection of the catecholaminergic neurotoxin 6-hydroxydopamine (6-OH DA) into the LC, or by i.c.v. administration of 6-OH DA or, by systemic administration of N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4). Among them, systemic DSP-4 (Fritschy & Grzanna, 1992; Fornai et al., 1996, 2001; Weinshenker et al., 2008) and 6-OH DA microinjection are considered those more specific in terms of LC lesion, while i.c.v. 6-OH DA administration has been shown to induce LC less consistently and to affect also other catecholaminergic neurons (Breese & Traylor, 1971). LC lesion by 6-OH DA microinfusion was neither shown to induce any modification in BBB permeability to albumin (Harik & McGunigal, 1984), nor its lesion by DSP-4 induced significant modification of horseradish peroxidase leaking (Tengvar, Pettersson, Mohammed, & Olsson, 1989). *In vitro*, in preparations of bovine brain microvessels, Borges et al., confirming what they had already observed *in vivo* by LC stimulation (Sarmento et al., 1994), showed that bath application of NA or phenylephrine increases the permeability to fluorescein (Borges, Shi, Azevedo, & Audus, 1994; Sarmento, Borges, & Azevedo, 1991). They also observed an increase in endothelial pinocytosis by electron microscopy (Sarmento et al., 1991) and since vincristine, which is known to hamper pinocytic vesicles organization, prevented such an effect, they even hypothesized that this was a key mechanism through which LC affects BBB permeability (Borges et al., 1994).

Tengvar had also shown that, acutely or subacutely after DSP-4 administration, indirect signs of interstitial edema could be observed (Tengvar et al., 1989). Previously, Harik (1986) had shown in rats that LC lesion by 6-OH DA induces a significant reduction of intracellular concentration of radiolabeled Ouabain, which is a substrate of Na^+/K^+pump (a substrate of the ionic pump; Harik, 1986). However, apparently, such an alteration of Ouabain binding sites was not paralleled by alterations of tissue content of Na^+, K^+, or water under steady-state conditions, and these parameters were only slightly affected by the induction of ionic disequilibrium (namely hyperkaliemia and hyponatremia; Moufarrij & Harik, 1989). Thus, the

TABLE 1 Effects of LC-NA manipulation on blood–brain barrier

Study	Animal model	Condition	LC manipulation	Substances acting on NA system	BBB item assessed	Assay used	Results
Raichle et al. (1975)	Rhesus monkeys	Anesthetized	LC carbachol microinfusion	–	Water permeability	Radiolabeled water ($H_2{}^{15}0$)	Increased permeability
			–	Phentolamine			Reduced permeability
Preskorn et al. (1981)	Rats	Anesthetized	–	Amitriptyline	Water permeability	^{14}C-butanol	Increased permeability
				Lithium			
				ECS			Reduced permeability
Swann, Crawley, Grant, and Maas (1981)	Rats	Brain sample (*in vitro*)	–	Piperoxane	Na^+/K^+ pump	Ouabain	Increased pump activity
Swann, Grant, et al. (1981)	Rats	Anesthetized	LC electrical stimulation	–	Na^+/K^+ pump	Ouabain	Increased pump activity
			–	Piperoxane			
Ben-Menachem, Johansson, and Svensson (1982)	Rats	Acute hypertension (angiotensin)	6-OHDA microinfusion	–	Albumin permeability	^{125}I-labeled albumin	Increased albumin leakage
		Acute hypertension (NA)					
Harik and McGunigal (1984)	Rats	Acute hypertension (angiotensin)	6-OHDA microinfusion	–	Albumin permeability	^{125}I-labeled albumin	No changes
		Acute hypertension (NA)					Increased albumin leakage
		Seizures					Increased albumin leakage
Preskorn, Kent, Glotzbach, Irwin, and Solnick (1984)	Rats	TBZ	–	Amitriptyline	Water permeability	^{14}C-butanol	Increased permeability
Ginsberg, Busto, and Harik (1985)	Rats	Status epilepticus (hypertension prevented by arterial withdrawal)	6-OHDA microinfusion	–	Water permeability	^{14}C-butanol	No changes
Harik (1986)	Rats	Brain sample (*in vitro*)	6-OHDA microinfusion	–	Na^+/K^+ pump	Ouabain	Increased pump activity
Nag and Harik (1987)	Rats	Acute hypertension (angiotensin)	6-OHDA microinfusion	–	BBB permeability	HRP	No changes
		Acute hypertension (NA)					Increased permeability
Moufarrij and Harik (1989)	Rats	Hyperkaliemia	6-OHDA microinfusion	–	Na^+/K^+ pump	Na+/K^+ concentration	No ionic unbalance
		Hyponatremia					

(Continues)

TABLE 1 (Continued)

Study	Animal model	Condition	LC manipulation	Substances acting on NA system	BBB item assessed	Assay used	Results
Tengvar et al. (1989)	Mice	No stress	Systemic DSP-4	–	BBB permeability	HRP	No changes
					Water permeability	Brain edema	Increased edema
Sarmento et al. (1991)	Rats	Anesthetized	–	NA	BBB permeability	NaF/pinocytosis	Increased extraction of NaF and pinocytosis
				Phenoxybenzamine			Reduced extraction of NaF and pinocytosis
Borges et al. (1994)	Bovine	Brain endothelial cells monolayer	–	NA	BBB permeability	NaF/pinocytosis	Increased extraction of NaF and pinocytosis
				Phenylephrine			Increased extraction of NaF and pinocytosis
				Clenbuterol			Reduced extraction of NaF and pinocytosis
Sarmento et al. (1994)	Rats	Anesthetized	LC electrical stimulation	–	BBB permeability	NaF/pinocytosis	Increased extraction of NaF and pinocytosis[a]
Chi, Wang, Chang, and Weiss (1998)	Rats	Anesthetized	–	Isoproterenol	BBB permeability	^{4}C-alfa-aminoisobutyric acid	Increased permeability
				Timolol			Decreased permeability
Dunn-Meynell, Hassanain, and Levin (1998)	Rats	TBI	–	Prazosin	Recovery of BBB permeability	Brain edema	Increased edema
Pavlasek et al. (1998)	Rats	Anesthetized	LC electrical stimulation	–	BBB permeability	Mannitol	Increased permeability
Kalinin et al. (2006)	Rats	No stress	Systemic DSP-4	–	BBB integrity	TJ protein	Reduction of ZO1 and occludin, gliosis

[a]Phenoxybenzamine administration totally prevent the effect of LC stimulation on BBB, while when pindolol (beta-antagonist) was delivered, LC-related effects increased.

Abbreviations: BBB, blood–brain barrier; Carbachol, muscarinic agonist, which stimulates LC cells; Clenbuterol, beta-agonist; DSP-4, N-2-chloroethyl-N-ethyl-2-bromo-benzylamine hydrochloride; ECS, electroconvulsive shock; HRP, horseradish peroxidase; Isoproterenol, beta-agonist; NA, noradrenaline; NaF, sodium fluorescin; Phenoxybenzamine, alpha receptor blocker; Phentolamine, alpha-receptor blocker; Phenylephrine, alpha-agonist; Piperoxane, alpha2- antagonist (NA neurons activator); Prazosin, alpha-blocker; TBI, traumatic brain injury; TBZ, tetrabenazine, noradrenaline, and dopamine inhibitor; Timolol, beta-antagonist; 6-OHDA, 6-hydroxidopamine.

abovementioned pieces of evidence suggest that LC-NA may concur in modulating Na^+/K^+ pump activity, but noradrenergic impairment alone is not sufficient to impair transmembrane ionic balance, being this ionic pump under the control of multiple systems (Harik, 1986; Moufarrij & Harik, 1989).

Finally, the molecular and structural effects of LC on BBB have been recently assessed. Kalinin et al. have shown that LC lesion by DSP-4 affects the expression and assembly of TJ proteins, as they showed a reduced synthesis of ZO1 and occludin, together with a decreased production of their mRNA (Kalinin et al., 2006). In parallel, they also observed marked astrogliosis which they did put in relation (though neuroinflammation) with the abovementioned BBB disruption (Kalinin et al., 2006). Concerning structural alterations, in 2009 Steinle et al. evaluated retina capillaries in mice transgenic for the dopamine beta-hydroxylase gene (i.e., with a congenital reduction of NA synthesis), and found an increase in BM thickness and PC ghosts (Steinle, Kern, Thomas, McFadyen-Ketchum, & Smith, 2009).

Aside from the abovementioned few studies assessing the effects of LC lesion or LC stimulation *in vivo* (or NA application to EC culture) in basal conditions, some authors assessed also the effects of AR agonist/antagonist administration, and nonphysiological conditions. These data gave contradictory results, which are likely due to several biases: for instance, some authors assessed the effects of LC manipulation after traumatic brain injury, or after the induction of hypertensive state and/or prolonged seizures (Table 1). Other authors administered to rats NA i.c.v., assuming that this could be considered as a tool for reproducing LC stimulation; again, several authors administered systemically or i.c.v. AR agonists or antagonists, but the net effect on LC activity in all of these studies is of difficult extrapolation (Table 1). In particular, concerning the effects of AR modulation, LC neuron perikarya or terminals bear auto-receptors with an excitatory or inhibitory effect, which are differently modulated by different NA concentrations. Thus, it is very difficult to get a clear idea from these studies; their main results are reported in Table 1.

4.2 | Effects of LC activity on CBF and neurovascular coupling

Also concerning the role of LC on CBF, several experimental approaches, with sometimes confusing results, have been used in the last decades (Table 2). Even in this case, only a few studies used a plain experimental design, that is, the analysis of the effects of LC lesion or LC direct stimulation in physiological conditions. In the latter studies, LC lesion generally increased CBF. In particular, in rats in which LC had been lesioned, CBF was globally increased (Bates, Weinshilboum, Jean Campbell, & Sundt, 1977; Dahlgren, Lindvall, Sakabe, Stenevi, & Siesjö, 1981; Ramana Reddy, Yaksh, Anderson, & Sundt, 1986). Intracerebroventricular infusion of 6OH DA in basal conditions gave more conflicting results, as one study in rats showed a 45% CBF increase (Onesti, Strauss, Mayol, & Solomon, 1989), while other ones did not show any CBF alteration, neither in rats (Edvinsson, Hardebo, & MacKenzie, 1977; Kobayashi et al., 1991) nor in cats (Ramana Reddy et al., 1986).

On the other hand, when LC has been directly stimulated, it has been shown, almost invariably and independently of the specific protocol and experimental model used, a global CBF significant reduction (Buchweitz, Edelman, & Weiss, 1985; de la Torre, Surgeon, & Walker, 1977; Goadsby & Duckworth, 1989; Katayama, Ueno, Tsukiyama, & Tsubokawa, 1981; Ohta et al., 1991; Raichle et al., 1975).

Thus, in basal conditions, there is converging evidence for the role of LC activity in decreasing global CBF.

This effect has been interpreted to play a key role in the neurovascular coupling occurring in specific cortical areas during physiological activation. In fact, it is known that LC-NA could differentially modulate neuronal networks, depending on their activity state (Devilbiss & Waterhouse, 2004), and in line with this, it has been proposed that a similar regionally specific function could be exerted also on neurovascular coupling.

A potential interpretation of the abovementioned results on LC effects on global CBF, reconciling them with the CBF redistribution occurring in specifically activated areas, has been given recently by Bekar, Wei, and Nedergaard (2012). In an experimental setting in which the effects of LC lesion by DSP-4 were assessed during sensory stimulation in rats, the authors suggested that LC-NA contribution to neurovascular coupling may represent a drive for CBF optimization. In this *scenario*, lowering blood flow in "resting" cerebral regions allows its selective increase in those brain areas which are mostly activated. In fact, the functional hyperemia occurring after limb stimulation, which was maximal in the somatosensory cortex in control rats, involved a much larger cortical area and lasted significantly longer after LC lesion by DSP-4 (Bekar et al., 2012). Bekar and colleagues interpreted their data speculating that, while LC activation occurring during somatosensory stimulation exerts a widespread vasoconstrictive effect on the cortical mantle, such an effect does not occur at the level of the areas activated by that specific stimulation, since at that level the local recruitment of NVU driven by neuronal activation could bypass the vasoconstrictive effects of LC-NA (Bekar et al., 2012).

In 2013, Toussay et al. further explored such mechanisms, by detailing how NVU and LC-NA interact at the microvascular level. They observed that LC projections target a large cohort of GABAergic interneurons which synthesize also other co-transmitters, namely PV, somatostatin, or NO (Toussay, Basu, Lacoste, & Hamel, 2013). These interneurons are known to actively participate in modulating neurovascular coupling, by exerting different effects also depending on the co-neurotransmitter released and on the activity state of the neural circuit (Anenberg et al., 2015; Duchemin, Boily, Sadekova, & Girouard, 2012; Urban, Rancillac, Martinez, & Rossier, 2012). Thus, local interneuron networks may significantly contribute to the coupling of LC-NA signal with local CBF demand, integrating multiple neurovascular inputs (Bekar et al., 2012; Toussay et al., 2013).

Several groups tried to address the role of LC on CBF also indirectly, by administering NA i.c.v and assessing, in some instances,

TABLE 2 Effects of LC-NA manipulation on cerebral blood flow

Study	Animal model	Condition	LC manipulation	Substances acting on NA system	Drug delivery	Results
Rosendorff and Cranston (1971)	Rabbits	Anesthetized	–	NA	Intrahippocampal	Low dose of NA: increase in CBF; high dose of NA: reduction of CBF
Raichle et al. (1975)	Rhesus monkeys	Anesthetized	LC carbachol infusion	–	Focally into LC	Global reduction of CBF
			–	Phentolamine	Intracerebroventricularly	Global increase in CBF
MacKenzie, McCulloch, and Harper (1976)	Baboons	Anesthetized	–	Reserpine	Intra-carotid	Global increase in CBF
			–	Propranolol	Intra-carotid	Global reduction of CBF
Bates et al. (1977)	Cats	Anesthetized	Stereotaxic lesion	–	–	Higher CBF in resting state
Edvinsson, Hardebo, MacKenzie, and Owman (1978)	Rats	Anesthetized/BBB disrupted by hypertonic urea	–	NA	Intra-venous	Global increase in CBF
				Propranolol	Intra-venous	Global reduction of CBF
Edvinsson, Lacombe, Owman, Reynier-Rebuffel, and Seylaz (1979)	Rats	Anesthetized/BBB preserved	–	NA	Intra-venous	Global reduction of CBF
			–	Phentolamine	Intra-venous	Prevented NA-related reduction
			–	Isoprenaline	Intra-venous	Global increase in CBF
			–	Propranolol	Intra-venous	Prevented isoprenaline-mediated increase
Dahlgren et al. (1981)	Rats	Paralyzed	LC stereotaxic lesion	6-OHDA	Focally into NA ascending bundle	No changes were detected
Katayama et al. (1981)	Cats	Anesthetized	LC electrical stimulation	–	–	Reduced CBF in resting state
Buchweitz et al. (1985)	Cats	Anesthetized	LC electrical stimulation	–	–	Reduced CBF in resting state
Ramana Reddy, Yaksh, Anderson, and Sundt (1986)	Cats	Anesthetized	LC stereotaxic lesion	–	–	Alteration of CBF changes CO_2 dependent
Goadsby and Duckworth (1989)	Cats	Anesthetized	LC electrical stimulation	–	–	Reduced CBF in resting state
Ohta et al. (1991)	Cats	Anesthetized	LC electrical stimulation	–	–	Reduced CBF in resting state
Bekar et al. (2012)	Mice	Anesthetized	Systemic DSP-4	–	–	LC optimizes coupling of cerebral blood volume with oxygen demand
Toussay et al. (2013)	Rats	Anesthetized	Systemic DSP-4	–	–	LC modulation of vascular coupling requires the activation of interneuronal networks

Abbreviations: Carbachol, muscarinic agonist, which stimulates LC cells; CBF, cerebral blood flow; DSP-4, N-2-chloroethyl-N-ethyl-2-bromo-benzylamine hydrochloride; Isoprenaline, beta-agonist; NA, noradrenaline; Phentolamine, alpha-receptor blocker; Propranolol: beta-antagonist; Reserpine, NA releaser; 6-OHDA, 6-hydroxidopamine.

the modulatory effects of modulation of different AR (see Table 2); these data are in our opinion of difficult interpretation due to the several biases already described in the previous paragraph concerning the BBB.

In conclusion, it can be generalized that LC-NA plays an important role in neurovascular coupling, by interacting with several local and global pathways. LC-NA likely contributes to functional hyperemia by reducing CBF in the less active regions, allowing its selective focal increase only in those neuronal circuits which are specifically activated at a particular moment.

5 | EVIDENCE FOR LC DEGENERATION IN AD

In the last decades, a large amount of data has been obtained in animal models and patients, linking the occurrence of LC alterations/degeneration with AD pathogenesis. The first studies date back to the early 1980s, when it was shown the occurrence of a marked LC cell loss in the brain of subjects with AD (Bondareff, Mountjoy, & Roth, 1982; Mann, Yates, & Marcyniuk, 1984; Tomlinson, Irving, & Blessed, 1981). The strong involvement of LC was confirmed also in more recent studies including only patients with less severe and better-defined AD (Zarow, Lyness, Mortimer, & Chui, 2003), up to very recent ones performed in patients with MCI due to AD (Kelly et al., 2017). In fact, Kelly et al. have clearly shown by sophisticated stereological analysis of brains of patients with AD or MCI due to AD, a significant neuronal loss in the LC compared with healthy controls, which correlates with the severity of cognitive alterations (Kelly et al., 2017).

5.1 | Potential mechanisms of LC degeneration in AD

While early reports hypothesized the occurrence of a progressive mild degeneration of LC during physiological aging (Manaye, McIntire, Mann, & German, 1995), recent data obtained by stereological analysis clearly demonstrated that the LC does not show any significant cell loss during aging (Theofilas et al., 2017). Therefore, rather than a natural consequence of aging, LC degeneration should be considered a pathological finding. In the last years, several authors addressed the issue of the potential mechanisms through which LC degenerates in AD. With this regard, a groundbreaking discovery was done in 2011 by Braak and colleagues (2011), who analyzed hundreds of brains of subjects showing different degrees of AD pathology, and showed that a progressive accumulation of hyperphosphorylated Tau (p-Tau, which the authors themselves called "pre-tangle inclusions") within LC precedes by years the occurrence of NFT deposits in the entorhinal cortex (which was classically considered, up to that study, as the first site involved by Tau pathology in AD). In particular, Braak et al. (2011) showed the accumulation of p-Tau within LC in brains which did not show yet Tau-related pathology in the trans-entorhinal region [which represents the so-called stage I of NFT pathology scale, according to the previous Braak's classification of NFT pathology of AD (Braak & Braak, 1991)].

Based on the severity of brainstem involvement by p-Tau accumulation, Braak and colleagues even described a progressive staging score ("a", "b", "c") up to early cortical recruitment (stages "1a" and "1b"), before the occurrence of NFT stage I. In detail, these stages were defined according to an increasing p-Tau neuronal staining, ranging from its accumulation only at the level of the LC proximal axon close to the soma (stage a), up to diffuse accumulation in LC neurons and, to a milder degree, also in non-thalamic nuclei such as raphe or magnocellular nuclei of basal forebrain neurons (stage c; Braak et al., 2011).

Interestingly, the fact that tau alterations in the brain of patients precedes by several years the earliest signs of amyloid pathology challenged dramatically the classical "amyloid cascade hypothesis" postulating that amyloid-pathway impairment precedes or may even trigger tau pathology in AD patients (Hardy & Higgins, 1992; Jack et al., 2010). A potential interpretation of these data, reconciling with previous hypothesis might be that early tau pathology might predispose to beta amyloid accumulation, which in turn might further exacerbate Tau pathology. In this context LC functional impairment might play a critical role in the onset of these events cascade.

Very recently, what observed by Braak et al. (2011) has been reproduced at least in part in AD rat model by Rorabaugh et al. (2017), who showed that in transgenic rats expressing both the Swedish APP and presenilin-1 mutations, there is a progressive alteration of LC which accumulates p-Tau and shows progressive NE fiber loss. Furthermore, the learning impairment observed in these rats was reversed by LC chemogenetic activation (Rorabaugh et al., 2017).

There are several potential reasons explaining a peculiar frailty of LC neurons, in parallel with its proneness to accumulate abnormal Tau protein, and most of them have been reviewed in detail recently by Weinshenker (2018). At least some of them are worth mentioning here. LC neurons fire tonically, and such tonic activity relies upon activation of voltage-dependent Ca++ channels. This may lead to excessive intracellular Ca++ levels which may be associated with oxidative stress (Cho, 2014; Sanchez-Padilla et al., 2014). Furthermore, this tonic activity causes persistently high NE turnover, which can also, by itself, increase NE-derived oxidative species, overwhelming LC antioxidant capabilities. This might be the case of the metabolite 3,4-dihydroxyphenylglycolaldehyde (DOPEGAL), which has been shown in the LC of AD patients (Burke et al., 1999) and has been recently shown to play a critical role in LC degeneration occurring in AD (see below), similar to what shown for the DA metabolite 3,4-dihydroxyphenylacetaldehyde for substantia nigra pars compacta DA neurons in Parkinson's disease (Colzi et al., 1996; Fornai et al., 2000; Kumar, Hsu, Lakshmi, Gillespie, & Burke, 2019).

Additional specific features of LC concurring to its frailty might be the thinness and extreme length of its unmyelinated axonal fibers (Nagai et al., 1981). Again, neuromelanin, an endogenous by-product of NE catabolism which is a chelator of metal ions, which

accumulates within LC neurons up to 60 years and is considered as a buffer of endogenous toxic compounds (Zecca et al., 2004), may later become itself a releaser of toxic species (Pamphlett, 2014).

A very recent study disclosed potential mechanisms by which LC may be particularly prone to Tau-related pathology, dysfunction and degeneration (Kang et al., 2020). LC NE is metabolized by the enzyme monoamine oxidase-A (MAO-A) which is particularly represented within LC neurons. The product of MAO-A activity on NE is DOPEGAL, which is produced exclusively in tonically active noradrenergic neurons. Within suffering LC neurons, a compensatory LC hyperactivity can occur, which produces an increased ratio of NE turnover, which is reflected by intraneuronal increase in DOPEGAL/NE ratio and DOPEGAL production is associated with intense oxidative stress, which may contribute to neuronal death (Burke et al., 1999; Weinshenker, 2018). Kang et al., in large study in which they profited of different techniques, of different transgenic animals *in vivo* and different cell lines *in vitro*, showed that DOPEGAL might be key in triggering tau-related LC modifications (Kang et al., 2020). In particular, they showed that: (a) DOPEGAL triggers, both *in vivo* and *in vitro*, the aggregation of Tau fibrils, (b) cell death-induced by DOPEGAL requires the expression of Tau, (c) MAO-A-mediated DOPEGAL production within LC neurons activates the lysosomal enzyme asparagine endopeptidase (AEP), which mediates Tau cleavage in protein sites which favor its hyperphosphorylation and pathologic aggregation, (d) preventing DOPEGAL metabolism by aldehyde reductase inhibition is deleterious to the neuron. Kang et al. also showed that in P301S mice, which bear the human tau P301S mutation, cognitive alterations and tau pathology requires the presence of NE (Kang et al., 2020). Interestingly, in specimens collected from AD autopsies, they showed an excess of activated AEP in LC in parallel with hyperphosphorylated and aggregated Tau. Finally, they demonstrated that AEP contributes to the propagation of pathological Tau, from the LC to the forebrain (Kang et al., 2020).

Pre-tangle Tau accumulation the LC is not *per se*, however, an index of degeneration; actually, it has been repeatedly shown that LC neurons bearing p-Tau do not necessarily degenerate, as LC neuronal loss can be observed only from around Braak NFT stage II-III (Theofilas et al., 2017). P-Tau may, however, cause already by itself functional impairment of LC neurons, as it can cause an impairment of axonal cytoskeleton and axonal transport, leading to altered pattern of release of NE in target regions; the release of NE is finely tuned, and regulated also by the strong afferents from different sensory inputs and collaterals of surrounding neurons; such afferents are altered early in the course of this hypothetical cascade, as shown by reduced synaptophysin-positive perineural dots (Andrés-Benito et al., 2017), making surviving LC cells less efficient as neuromodulators of target regions (as witnessed by altered expression of AR in target limbic regions, (Andrés-Benito et al., 2017). Again, it has been proposed, according to a "two-hit" model, that LC neurons bearing excessive p-Tau may degenerate when an additional mild neuronal insult, which would be ineffective in normal LC neurons, occurs (reviewed in Chalermpalanupap, Weinshenker, & Rorabaugh, 2017).

In summary, LC is impaired early in the course of AD, this is likely to be mainly related to Tau pathology, whose accumulation inside LC neurons is facilitated by LC-specific features themselves; eventually this is associated also with LC neuronal degeneration and cell loss, which is a constant feature in AD patients.

5.2 | Potential role of LC degeneration in AD pathogenesis

Experimental studies on animal models had further explored the possible role of LC degeneration in AD pathogenesis, and how it may contribute to its main pathological processes, such as amyloid deposition, neuroinflammation, and tau pathology. In 2006, Heneka and colleagues assessed the effect of LC lesion in a mouse model of AD, and tried to dissect the mechanisms through which LC degeneration could contribute to amyloid accumulation in AD (Heneka et al., 2006). They performed a study using APP23 transgenic mice, which is a widely used animal model of AD in which there is an expression of a mutated form of APP causing autosomal-dominant AD in patients (Sturchler-Pierrat et al., 1997), in which LC degeneration was induced by intraperitoneal DSP-4 injection. Animals were then sacrificed after 6 months, and authors observed the occurrence of a marked increase in amyloid burden in cortical and subcortical areas in LC-lesioned APP23 mice, when compared to APP23 with an intact LC. Interestingly, in areas that are not innervated by LC, such a difference could not be observed; the latter finding was interpreted by Heneka as a further evidence supporting the contribution of LC degeneration to amyloidogenesis. Furthermore, Heneka et al. also observed that such an increased amyloid plaque deposition was paralleled by increased neuroinflammation and neuronal damage in the brain of LC-lesioned animals (Heneka et al., 2006). Thus, in 2010 the same group further assessed the link between LC and neuroinflammation (Heneka et al., 2010), profiting of another AD animal model, namely APP/PS-1 transgenic mice (Moechars et al., 1999). They observed that the stimulation of LC-NA produced a strong reduction of cytokine levels, together with increased activation of microglia and promotion of Aβ phagocytosis by microglia themselves. Conversely, they found that lesioning LC with DSP-4 caused a dramatic reduction of microglial activation and phagocytosis, as well as an increased release of pro-inflammatory cytokines; finally, they observed that restoring brain NA reversed such inflammation-related alterations (Heneka et al., 2010). Heneka and colleagues interpreted these findings as proof of the anti-inflammatory effect of LC-NA and, at the same time, they suggested that neuroinflammation could be another mechanism by which LC degeneration could contribute to AD pathogenesis (Heneka et al., 2010).

Nonetheless, as observed by Braak et al. in 2011, LC pathology in AD starts earlier than amyloid deposition and neuroinflammation activation, with the occurrence of the progressive accumulation of p-Tau in LC neurons (Braak et al., 2011). Thus, in 2015 Iba and colleagues performed a study in PS19 tau transgenic mice, specifically designed to assess the potential role of the early occurrence

of tau accumulation into the LC on the occurrence of Tau pathology in the cortex in AD; they injected synthetic tau fibrils into LC, and showed its propagation through noradrenergic projections, eventually involving cortical and subcortical regions. Such a spreading of Tau pathology was interpreted as a piece of evidence supporting the transneuronal spreading of tau-related tangles which had been already hypothesized by Braak et al. (2011), and as a proof of the potential role of LC as a starting point for tau pathology in AD (Iba et al., 2015).

Moreover, the lesion of LC *per se* could contribute, aside from its role in Tau propagation, to increase Tau accumulation in AD, as shown recently by Chalermpalanupap and colleagues in P301S tau transgenic mice. After the administration of DSP-4, they observed that, compared with intact mice, LC-lesioned mice showed earlier cognitive impairment, together with an increased burden of tau pathology, hippocampal neuroinflammation, and neurodegeneration (Chalermpalanupap et al., 2018).

Evidence of LC involvement in AD has been obtained also *in vivo* in humans. In the last two decades, several groups assessed LC by MRI, profiting of specific sequences that highlight neurons containing neuromelanin, such as those belonging to the LC (Liu et al., 2017; Sasaki et al., 2006). This field of MRI research is still in progress, as more sophisticated acquisition/postprocessing approaches applied to MRI data are being developed by several groups worldwide (Keren et al., 2015; Liu et al., 2017), but in any case some LC-MRI studies have already been performed in AD patients, confirming, *in vivo*, the significant involvement of LC in AD (Betts, Cardenas-Blanco, et al., 2019; Olivieri et al., 2019; Takahashi et al., 2015). Indeed, LC-MRI is starting to be considered even as one of the most promising tools to assess early degenerative phenomena in asymptomatic subjects at-risk for AD (Betts, Kirilina, et al., 2019). It is worth mentioning that also in the field of nuclear medicine specific noradrenergic tracers that have been recently developed might provide useful data *in vivo* on the state of LC terminals in patients (Knudsen et al., 2018).

Whatever be the mechanism by which LC early degenerates, as abovementioned it is nowadays widely accepted its early involvement in the course of AD pathogenesis. In the previous paragraphs, we have detailed some aspects of NVU which are influenced by LC terminals. In the next paragraph, we will briefly describe the involvement of NVU in AD pathogenesis and, eventually, we will discuss, in the last part of the review, how the early degeneration of LC terminals in the course of AD pathogenesis might contribute to such pathogenesis itself by significantly interfering with different aspects of the NVU.

6 | EVIDENCE AND MECHANISMS OF NVU ALTERATION IN AD

Significant involvement of NVU has been shown to play an important role in neurodegenerative diseases (NDD), and particularly in AD.

It is still unclear whether the vascular pathology should be considered mainly as a cause or as a consequence in AD pathophysiology, as there are data from histopathological studies, experimental models, and genetic evaluations, which support both hypotheses. For instance, according to the "two-hits" hypothesis for AD, two sequential stages occur, which could establish a vicious circle (Zlokovic, 2011); first, microvascular damage may impair neuronal oxygen supply and BBB exchanges, exerting detrimental effects on neuronal metabolism. Thus, protein clearance pathways could be altered, leading to parenchymal β-amyloid accumulation, neuronal death, and even to further vascular impairment due to β-amyloid accumulation also into the vessel walls (known as cerebral amyloid angiopathy, CAA).

NVU alteration might concur to AD pathogenesis through multiple mechanisms, related to the different parts and cells which compose the NVU itself. The damage of endothelium can cause BBB disruption, jeopardizing the selectivity of physiological substances exchange and interfering with protein clearance pathways. At the same time, an impairment of neurovascular coupling may cause relative hypoperfusion and relative hypoxia in areas with high energy demand, which might, when prolonged enough, potentially induce cell suffering. Thus, the accumulation of pathological proteins may cause neurotoxicity, which might be associated also with neuroinflammation and reactive gliosis. Altogether, these mechanisms might result in neural degeneration up to neuronal death and be key in the pathogenesis of NDD (Iadecola, 2017). In the following paragraphs we will dissect the potential role in AD of the impairment of every single component forming the NVU.

6.1 | BBB disruption

Transcytosis dysregulation and water-ionic balance failure are key features of BBB damage occurring in AD (Yu, Ji, & Shao, 2020). Loss of intercellular junctions, ECM alteration, and PC death are structural changes often observed in these conditions. Pericytes degenerate in AD and AD Tg models (Halliday et al., 2016; Sagare et al., 2013), and PC loss, among other functional consequences (Sweeney et al., 2016), significantly affects BBB permeability (Villaseñor et al., 2017).

Concerning AJ and TJ, in AD, levels of occludin, claudin, and ZO-1 are strongly reduced in capillaries in parallel with the increasing burden of CAA (Yamazaki & Kanekiyo, 2017). β-amyloid and α-synuclein oligomers exert direct toxic effects on ECs, with specific harmful effects on the integrity of TJ and AJ (Costea et al., 2019). Again, the occurrence of neuroinflammation and plasmatic protein extravasation in ECM leads to the activation of metalloproteinase (MMP), which could target junctional proteins too (De Luca & Papa, 2017; Rosenberg, 2009). Moreover, ECs can suffer from a metabolic and energetic impairment, which may be due to inflammation, insufficient oxygen delivery and accumulation of toxic by-product, such as reactive species of oxygen (ROS) released by damaged mitochondria and activated glial cells

(Zlokovic, 2008). As a consequence, also ATP production within ECs decreases, and this might reduce Na^+/K^+ pump function, thus leading to alteration of cellular membrane potential and cytotoxic edema (de Lores Arnaiz & Ordieres, 2014).

Due to the abovementioned alterations, the endothelial barrier can be crossed by plasmatic proteins such as albumin, immunoglobulin, plasminogen, etc., which can accumulate in the ECM (Zlokovic, 2011), and it has been even proposed to represent the first "hit" in a "two-hit" hypothesis of AD pathogenesis (Zlokovic, 2011). Protein accumulation leads to interstitial edema, which by itself can cause further disruption of the NVU complex. Moreover, some plasma proteins could directly exert harmful effects on the NVU; for instance, plasmin facilitates ECM laminin degradation, fibrin enhances vascular damage, and thrombin has been shown to activate inflammatory pathways (Cai et al., 2017).

In particular, thrombin seems to interact with proteinase-activated receptors (PARs), which are G-coupled receptors expressed by neuron and glial cells (Junge et al., 2004). PARs may have an important role in mediating neurovascular damage in pathological conditions, since it has been observed that their inhibition could reduce ischemic damage in animal models (Rajput et al., 2014).

In AD, ECM degradation may play a key pathogenic role (Nelson, Sweeney, Sagare, & Zlokovic, 2016). Thickening of brain capillary BM and alterations of its protein composition have been shown in specimens from AD patients and AD animal models (Thomsen et al., 2017). These changes may contribute to Aβ accumulation, and to hindering oxygen and glucose delivery to inhabiting cells (Morris, Carare, Schreiber, & Hawkes, 2014). Further pieces of evidence supporting this hypothesis come from genetic studies. ApoE4 allele, the most important genetic factor linked to AD, is associated with increased ECM degeneration, metalloproteinase activation, and Aβ deposition, the latter being due to reduced efficiency of clearance pathways which might be mediated by low-density lipoprotein receptor-related protein 1 (LPR1; Tai et al., 2016). MEOX2 is a transcription factor that regulates vascular cell differentiation and remodeling in BBB. Patients suffering from AD express lower levels of MEOX2, and knockout mice develop cerebral endothelial hypoplasia with reduced brain perfusion (Wu et al., 2005).

Besides endothelium disruption and ECM degradation, PC death could represent a third key element in BBB breakdown. PC play a fundamental role in BBB homeostasis and CBF regulation, and their degeneration has been repeatedly reported in AD (Zlokovic, 2011). PC impairment could reduce the expression of occludin, claudin, and ZO-1, contributing to TJ and AJ damage (Bell et al., 2010). In AD it has been shown that Aβ1-42 oligomers could be toxic for PC (Winkler, Sagare, & Zlokovic, 2014), while in an APP-mouse model, worsening of both amyloid and microtubular pathology was observed after experimental PC depletion (Sagare et al., 2013). Recently, in patients affected by mild AD dementia, it was shown an association between the degree of BBB disruption and CSF concentration of soluble platelet-derived growth factor receptor, which is considered a reliable marker of PC degeneration (Nelson et al., 2016).

6.2 | Neurovascular uncoupling

Several pieces of evidence support a possible causative role of CBF impairment in NDD (Cai et al., 2017); hypoperfusion and subsequent relative hypoxia could be responsible for neuron/glial cell death, thus contributing to the neurodegenerative process. In 2005, the Rotterdam study showed a reduced CBF in AD patients, and that patients with lower CBF had an increased risk of developing cognitive decline compared with those with higher CBF (Ruitenberg et al., 2005).

In this *scenario*, coupling impairment is not only due to concomitant vascular pathology, for example atherosclerosis, but also to degenerative phenomena directly involving the NVU. Neurovascular coupling seems to be affected at multiple levels; as already reported above, ECs and PC are altered in AD. Besides their role in BBB integrity, those cytotypes play a key role in the CBF regulation at capillary level; this is likely to contribute to brain microcirculation dysregulation, depriving neurons and glial cells of appropriate oxygen and glucose supply in a condition which is called *oligoemia* (Zlokovic, 2011).

Oligoemia interferes with normal cell metabolism, as it hinders normal mitochondrial functioning, ATP production, and protein synthesis and turnover. Impaired mitochondria release ROS, which might further alter neurovascular coupling; ROS are responsible for oxidative stress, to which ECs are very susceptible; furthermore, ROS react with NO, forming peroxynitrite and thus hindering NO-mediated vasodilation (Iadecola, 2004; Islam, 2017).

In AD, oligoemia may contribute to worsening Aβ accumulation into vessel walls (i.e., CAA); amyloid deposits weaken vascular walls, up to leading to capillary rupture and/or occlusion, and thus predisposing to microhemorrhages or focal ischemic damage. Moreover, it has been shown also that β-amyloid can cause overactivation of SMC in brain arterioles; hypercontractile SMC reduce CBF via aberrant arteriolar vasoconstriction, thus further exacerbating tissue hypoperfusion (Nelson et al., 2016).

Therefore, in AD a global failure of NVU could be hypothesized, due to the involvement of its single components to varying degrees and resulting in hypoperfusion and cellular damage. This process (as already above proposed also for BBB) could lead to a vicious circle in which vascular damage enhances degenerative processes, which in turn can exacerbate NVU disruption.

6.3 | Glial cells and neuroinflammation

In the last decades, a huge amount of data has shown a key role of neuroinflammation in AD (Heneka et al., 2015). The neuroinflammation process is featured by the release of pro-inflammatory cytokines and chemokines, together with the activation of astrocytes and microglial cells (Ransohoff, 2016). Even though at least in part neuroinflammation processes can be seen as an attempt to contain the ongoing neurodegenerative process, it turns out that from a beneficial element, it could become an additional pathogenic factor for neurodegeneration (Shabab, Khanabdali, Moghadamtousi, Kadir, & Mohan, 2017). Cytokines like interleukin-1 (IL-1), interleukin-6 (IL-6),

and tumor necrosis factor-α (TNF-α) exert neurotoxic effects in several experimental paradigms (Becher, Spath, & Goverman, 2017); activated microglia releases harmful molecules like ROS, can impair synaptic plasticity, and induce axonal demyelination and degeneration (Chen, Zhang, & Huang, 2016); moreover, neuroinflammation can exacerbate accumulation of aberrant proteins, such as β-amyloid, phospho-tau, or α-synuclein (Ransohoff, 2016).

Regarding glial activation, it is worth mentioning the role of neuroimmune regulators (NIREGs). NIREGs are a group of functional-related proteins, which have anti-inflammatory properties and maintain microglia in resting state (De Luca et al., 2020). They are strategically placed on BBB, in order to balance inflammatory mechanisms related to neurovascular injury, especially preventing complement activation and regulating macrophage diapedesis (Hoarau et al., 2011). In case of BBB disruption, NIREGs modulate also the abovementioned PARs system; indeed thrombomodulin, which is considered to be a NIREG, inhibits PARs activation, thus reducing thrombin neurotoxicity (Niego, Samson, Petersen, & Medcalf, 2011). In AD, NIREGs dysfunction may be related to more severe neuroinflammation, making them interesting therapeutic target (Bedoui, Neal, & Gasque, 2018).

Aside from glial cells, ECs themselves could release inflammatory factors, such as IL-1, IL-6, and TNF-α, and promote the migration of the immune cells from the blood to the brain parenchyma (Grammas, 2011). At the same time, ECs are damaged by oxidative stress and cytotoxic released factors (Islam, 2017; Zlokovic, 2011), and, as already described, endothelium disruption *per se* has several negative consequences on BBB and neurovascular coupling. Moreover, chemokines released by ECs contribute to microglial activation. Microglia contribute to neuroinflammation and in particular to the response to aberrant protein accumulation; protein-engulfed and dysfunctional microglial cells are a typical feature of pathological samples in NDD (Heneka et al., 2015). Among others, also microglia have been linked to BBB breakdown: in fact, by releasing IL1 and TNF-α, they could lower EC expression of TJ and AJ protein complex; furthermore, microglia produces MMPs which degrades ECM components as well as TJ and AJ themselves (Thurgur & Pinteaux, 2019).

Concerning astroglial cells, their hyperactivation may also interfere with NVU functioning (Heneka et al., 2015). Reactive astrocytosis is a key feature of neuroinflammation, and may contribute to neuronal damage and protein accumulation; given their key role in NVU, astrocytes involvement in neuroinflammation could potentially have significant detrimental consequences on NVU itself (Colombo & Farina, 2016). In particular, reactive astrocytes are thought to be less efficient in mediating neurovascular coupling. Astrocytes dysregulation of NO production and downregulation of K+ channels have been described in AD model: the reduction of NO may hinder the vasodilation of capillaries, producing hypoperfusion and hypoxia, while deficiency of K+ channel could hamper K-dependent Ca+ release, an important signaling pathway of NVU (McConnell, Li, Woltjer, & Mishra, 2019). Furthermore, reactive astrocytes could have lost, at least in part, their supporting role on BBB; the end feet layer around endothelium appears disorganized and discontinuous in AD, and it could compromise BBB structural and functional homeostasis (Zlokovic, 2008). Moreover, reactive astrocytes show reduced expression of AQP4, with important consequences on water balance and ionic flow through BBB (Liu, Yang, Ju, Wang, & Zhang, 2018).

6.4 | Involvement of the GlyS

The GlyS defines those elements (primarily glial cells) which are key for the removal of catabolic by-products from the interstitial fluid (IF) of the brain (for reviews see Jessen et al., 2015; Plog & Nedergaard, 2018) and which have been already introduced in par. 2.3. Very recently it has been shown that GlyS impairment is likely to play a key role in AD pathogenesis (see below).

It is worth being mentioned here as the GlyS shares several components with NVU. In fact, most of the exchanges occurring between CSF and IF occur at the level of periarteriolar/capillary/perivenular level. CSF enters brain parenchyma through distal arterial PS and, at capillary level, through BM. Astrocyte end feet separate arteriolar and perivenular PS and capillary BM from IF, allowing the communication of these two compartments through AQP4 channels densely expressed on these. At the level of IF, both osmotic and hydrostatic pressures contribute to a bulk convection flow, which allows waste compounds to be removed from IF to venular PS, and from there to the cerebral venous lymphatic system (Plog & Nedergaard, 2018). GlyS plays a critical role in Aβ clearance from the IF (Iliff et al., 2012) together with other clearance pathways, involving the BBB (Cockerill, Oliver, Xu, Fu, & Zhu, 2018). Furthermore, recently it has been shown that GlyS function is strictly regulated by Sleep/wake (S/W) cycle (Iliff et al., 2012; Xie et al., 2013), and Aβ clearance fluctuates in parallel with GlyS along the cycle, being higher during sleep and lower in wakefulness (Xie et al., 2013). Remarkably, recently these S/W cycle-dependent phenomena have been suggested also in humans by Fultz et al. by MRI (2019).

7 | THE POTENTIAL INTERACTION OF LC AND NVU IN THE PATHOGENESIS AND COURSE OF AD

LC is likely to play a key role in AD pathogenesis. Its effects on NVU might contribute to that (Figure 2). Alterations of brain circulation have been repeatedly shown in AD. Cardiovascular risk factors are strongly associated with AD occurrence (Scheltens et al., 2016), and small vessel insufficiency often occurs in patients with AD (Iadecola et al., 2019; Snyder et al., 2015). Furthermore, CAA occurs in up to 80% patients with AD (Greenberg et al., 2020); it is featured by the frailty of brain vessels, due to amyloid accumulation at the level of the SMC, PC, and endothelium, and these alterations cause microhemorrhages and subcortical small infarcts, which, aside from being associated with higher cognitive impairment, cause by themselves severe neurological outcome (Greenberg et al., 2020). The

above-quoted macroscopic alterations massively involve the NVU. Recent exciting data have shown that in rats transgenic for AD-related proteins, an early iatrogenic lesion of cortical LC terminals induces the occurrence of CAA several months earlier than in rats with intact LC (Kelly et al., 2019).

Concerning the increase in white matter ischemic alterations occurring in AD, no specific studies have assessed thus far the contribution of LC degeneration, even though the NVU alteration described above may concur to it. However, it is worth mentioning that there are some experimental pieces of evidence that brain NA might

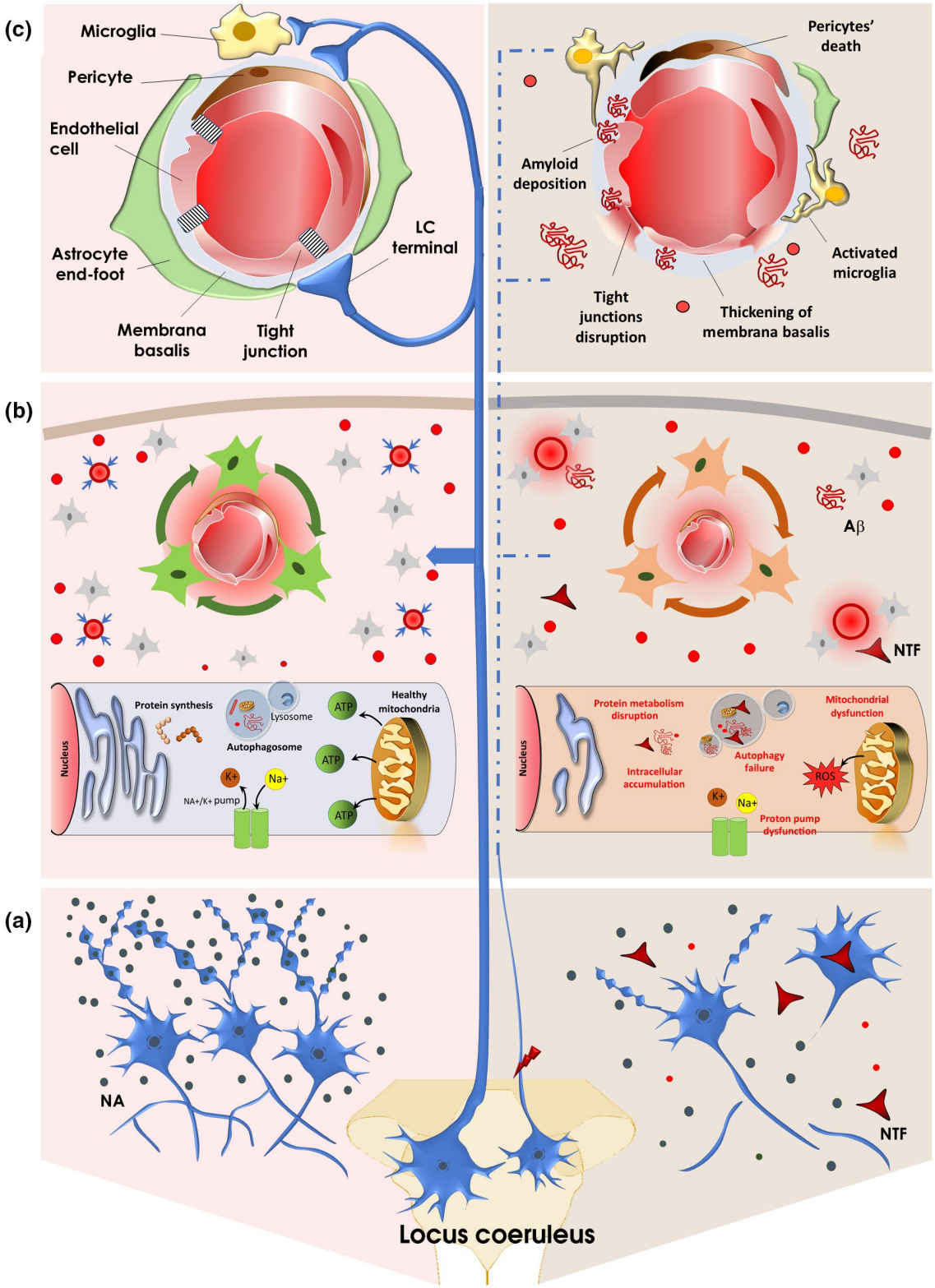

FIGURE 2 Roles of locus coeruleus (LC) on neurovascular coupling and blood–brain barrier homeostasis and possible pathological mechanisms involved in Alzheimer's disease (AD). In physiological conditions, LC neurons receive afferences from a variety of structures and send diffuse projections throughout the whole brain (Panel A, left). In AD, LC is the first brain structure to develop tau-pathology, and its degeneration occurs early during AD pathogenesis (Panel A, right). LC-NA is likely to play a key role in neurovascular coupling, by promoting the optimization of functional hyperemia (Panel B). In particular, when activated, LC produces a global reduction of cerebral blood flow (CBF) inducing arteriolar and capillary vasoconstriction; this provokes a shift of blood supply from inactive brain areas toward activated regions, since at the level of the latter regions LC signal is by-passed by local vasodilatory mechanisms, mainly driven by neurons and astrocytes (Panel B, left). In line with this, LC degeneration hampers the neurovascular coupling process, thus leading to oligoemia (Panel B, right). In particular, in this condition, the blood supply is insufficient in activated regions (relative oligoemia) whose neurons cannot fulfill the needed high metabolic demand; therefore, mitochondria stop working properly and start releasing reactive species of oxygen (ROS); at the same time, ATP production fails and protein synthesis and degradation breakdown (Panel B, right). Several data suggest that LC-NA is also important in BBB homeostasis, since it induces the expression of tight junctions (TJs), it regulates the BBB permeability to water and solute and it promotes transcytosis. Moreover, LC-NA is fundamental in modulating microglial cells, exerting an anti-inflammatory effect (Panel C, left). In AD, the protective contribution of LC-NA is lost and this may lead to BBB breakdown, worsening amyloid accumulation, promoting neuroinflammation and causing neuronal death (Panel C, right)

have beneficial effects on stroke extent and severity in animal models of brain ischemia and in stroke patients (Blomqvist, Lindvall, & Wieloch, 1985; Sternberg & Schaller, 2020).

In humans, both AD-related CAA and subcortical infarcts have been recently shown to occur already at a preclinical stage of AD (Yates et al., 2014), when LC degeneration processes have already started taking place.

In any case, as described in Section 6, aside from CAA and subcortical ischemic lesions, AD is associated with more subtle alterations of the NVU which might significantly concur to its severity and rate of progression. LC degeneration might participate in at least some of them.

In the next two paragraphs, we will describe, first how the different components of NVU involved in AD pathogenesis are likely affected by LC degeneration, and then how an early LC impairment may be key and associated with a chain of events leading to AD through NVU impairment.

7.1 | Specific aspects of neurovascular unit impairment occurring in Alzheimer's disease which might be affected by Locus Coeruleus loss

The LC plays a key role in the physiology of aspects of NVU which are key in AD pathogenesis. In particular, LC (a) modulates CBF and neurovascular coupling, (b) modulates BBB permeability, (c) strictly interacts with astrocytes and microglia in modulation neuroinflammation, (d) promotes the local synthesis of growth factors mainly through its effect on astrocytes (Figure 2).

All of those aspects are crucial in AD pathogenesis, and we describe below the potential involvement of LC in each one.

7.1.1 | Neurovascular coupling impairment

It has been proposed that LC modifies CBF by exerting a general vasoconstrictive effect, except for activated regions in which this vasoconstriction is by-passed by local vasodilatory mechanisms (mainly driven by neurons and astrocytes). This provokes a shift of blood supply from inactive brain areas toward activated regions, to fulfill their metabolic demand. On the contrary, the lack of such an LC-mediated effect causes a condition of relative oligoemia in areas with higher energetic demand during their physiological activation (Bekar et al., 2012). Neurons require a large amount of energy to work properly and to maintain their homeostasis; moreover, they completely depend on the blood supply, since they lack energy reserve. Thus, oligoemia is detrimental for neurons, not only when it occurs massively and acutely (such as during ischemic stroke), but also when occurring to a lower extent but during a prolonged period. When blood-derived oxygen supply is not sufficient, mitochondria stop producing ATP within a few minutes, and this interferes with several cellular pathways. In fact, protein synthesis and degradation are impaired and synaptic transmission becomes less efficient (de la Torre, 2017). Impaired mitochondria produce ROS, which cause oxidative damage of nucleic acid, lipids, and proteins. Due to ATP depletion, neurons can no longer maintain their antioxidant mechanisms, and damaged/misfolded proteins start to accumulate; at the same time, ATP deficiency hampers autophagic activity. Autophagy is an energy-demanding pathway that is important for neuronal homeostasis and housekeeping, as it removes damaged/misfolded proteins from the cytoplasm, delivering them to lysosomes (Menzies et al., 2017). Autophagy breakdown might be one of the key processes in AD pathogenesis (Li, Liu, & Sun, 2017), and oligoemia may contribute to it by depriving neurons of energy supply. Therefore, misfolded proteins progressively accumulate within neuronal cytoplasm, and interfere with intracellular processes, which are already compromised by oligoemia (Figure 2 – Panel B). Eventually, neurons can no longer bear such stressful conditions and die: this increases neuroinflammation and may contribute to the ongoing neurodegenerative process (de la Torre, 2017). As mentioned above, LC is likely to play a critical role in the oligoemic phenomena occurring in AD (Bekar et al., 2012).

7.1.2 | Blood–brain barrier dysfunction

LC is likely to contribute to BBB function, both modulating the expression of its structural proteins and regulating its permeability to water and ions.

It has been shown that the expression of TJ proteins (ZO1 and occluding) is under LC influence (Kalinin et al., 2006). Thus, LC degeneration may contribute to BBB disruption by hindering endothelial junctional complex assembly, thus provoking a pathological increase in its permeability.

It has also been shown that LC modulates BBB permeability to water and ions (Pavlasek et al., 1998; Sarmento et al., 1994; Tengvar et al., 1989).

In basal conditions, LC lesion has not been shown to increase BBB permeability to large molecules such as albumin (Harik & McGunigal, 1984). However, LC lesion induces a significant increase in BBB permeability to albumin after acute hypertension (Ben-Menachem et al., 1982; Nag & Harik, 1987; Harik & McGunigal, 1984; Table 1), and this might suggest a potential role of NA on BBB permeability to macromolecules in nonphysiological conditions. In line with this, Kelly et al., recently showed that LC terminal lesion significantly increases the leakage of albumin into brain parenchyma occurring in the Tg344-19 rat model of AD (Kelly et al., 2019) thus directly confirming the theoretical potential link between LC degeneration and AD-related BBB alteration.

Finally, LC significantly improves the activity of Na^+/K^+-ATPase and the lack of this effect might contribute to cell damage. In fact, proper functioning of Na^+/K^+-ATPase in the antiluminal side of ECs is required for maintaining appropriate ionic gradient for NA^+ and K^+ at the level of BBB, which in turn is important for the ionic balance of interstitial fluid and, eventually of neurons (Keep, Xiang, & Lorris Betz, 1993). Na^+/K^+-ATPase impairment contributes to cytotoxic edema of EC and ionic imbalance and this might represent one of the mechanisms by which LC degeneration causes EC degeneration, and thus BBB impairment (Zhang et al., 2013).

7.1.3 | Neurovascular unit-related neuroinflammation

LC might interfere with the neuroinflammation occurring in AD through its interaction with NVU components. NVU is key in neuroinflammation, and the concept itself of "neuroimmune system" (Abbas, Lichtman, & Pillai, 2017) includes the BBB, the astrocytes, the microglia, and the GlyS (Benveniste et al., 2018; Buckner, Luers, Calderon, Eugenin, & Berman, 2006). In AD, inflammatory factors impair the neurovascular unit at different levels and contribute to a vicious cycle involving a further increased release of cytokines by peripheral immune cells crossing an impaired BBB, and eventually leading to neuronal damage (Giorgi et al., 2019). Thus, neuroinflammation might be involved in the early steps of AD development, when LC is already significantly impaired; later on, at more delayed disease stages, neuroinflammation facilitates AP formation and enlargement and, at the same time, is induced by amyloid deposition itself (Cai, Hussain, & Yan, 2014; Penke, Bogár, & Fülöp, 2017). The role of NA in AD-related neuroinflammation has been directly addressed by studies specifically designed to explore this aspect, mainly by Heneka's group (e.g., Heneka et al., 2010), which showed a pro-amyloidogenic effect of NA loss, and the strong involvement in such phenomena of specific chemokines, of astroglial and microglial activation (e.g., Feinstein et al., 2002; Heneka, 2017; Heneka et al., 2002, 2003, 2010; Kalinin et al., 2007).

However, NA loss might significantly potentiate inflammation by acting mainly on NVU components, even before the onset of APs accumulation (for a detailed review, see Giorgi et al., 2019). For instance, among its potential anti-neuroinflammatory effects, NA induces the transcription of several anti-inflammatory genes in glia, such as heat shock protein 70 (Agac, Estrada, Maples, Hooper, & Farrar, 2018; Feinstein et al., 2002), IL-10, and peroxisome proliferator-activated receptor-γ (Klotz et al., 2003). In astrocytes, NA reduces MHC-II expression (Frohman, Vayuvegula, Gupta, & Van Den Noort, 1988), which is key in early steps of immune response; it also reduces the expression of MCP1 (Hinojosa, Caso, Garcia-Bueno, Leza, & Madrigal, 2013). In microglia, NA decreases NF-kB activity, and as a consequence, it also reduces the synthesis and expression of the pro-inflammatory TNF-α (Heneka et al., 2010). Astrogliosis itself, which is significantly increased by LC loss (Heneka et al., 2006; Kelly et al., 2019) is often associated with neuroinflammation, and it might also further interfere with BBB permeability, as reactive astrocytes could have lost at least in part their supporting role on BBB as shown by Kalinin et al. (2006). Furthermore, reactive astrocytes show reduced expression of AQP4, with important consequences on water balance and trans-BBB ionic flow (Liu et al., 2018); unfortunately to our knowledge, there are no studies in which the effects of LC lesion on the expression of AQP4 have been assessed.

7.1.4 | Growth factors

Finally, it is well known that NA strongly modulates growth factor (GF) synthesis and release by astrocytes (Follesa & Mocchetti, 1993; Furukawa et al., 1987; Juric et al., 2006; Kajitani et al., 2012; Krzan et al., 2001; Mocchetti et al., 1989). BDNF and FGF synthesized by astrocytes, aside from affecting neuron integrity, might interfere also on endothelial repair after different insults (Jin et al., 2019; Yang, Qiao, Meyer, & Friedl, 2009) and this might be one additional mechanism through which LC degeneration might interfere with BBB integrity. The release of FGF by astrocytes might also protect PC integrity and function under stressful conditions (such as hypoxia/ischemia) through an overexpression of platelet-derived growth factor receptor-β (Arimura et al., 2012; Nakamura et al., 2016).

7.2 | Locus coeruleus degeneration may trigger Alzheimer's disease pathogenesis also through neurovascular unit impairment

LC impairment occurs very early during AD development, even decades before the first appearance of the APs and NFT in the cortex,

and actually nowadays LC is considered as the first brain nucleus to be involved in AD. This has been fully acknowledged also in the revised staging of AD-related tau pathology proposed by Braak et al in 2011. They clearly showed that the first site of accumulation of p-Tau and NFT in AD is indeed at the level of LC terminals and neuronal bodies (Braak et al., 2011) and that the functional/structural impairment of LC due to tau pathology precedes by years the occurrence of cortical AD-related pathology and cognitive impairment (Braak et al., 2011; Figure 2 – Panel A).

The cortical pathology of AD itself might be significantly dependent on preexisting LC loss, both directly because of the lack of its neurotrophic effects and, indirectly, by its strong contribution to NVU failure.

In line with this, the following scenario in which LC is key in triggering and concurring to a chain of events involving the NVU might be hypothesized:

1. The progressive impairment of LC contributes to altering the regulatory mechanisms controlling precise neurovascular coupling. Thus, NA deficiency likely significantly contributes to a state of relative oligoemia in cortical areas physiologically activated, and, when persistent enough, such oligoemia itself may trigger a chain of events that significantly contribute to AD pathogenesis (de la Torre, 2017).
2. The first direct effect of relative oligoemia is the massive reduction of ATP production by mitochondria in all of the NVU components, from neurons, to supporting cells, up to the endothelium. This causes, among others, an impairment of Na^+/K^+-ATPase activity, which, in parallel is also directly affected by NA loss (Harik, 1986; Swann, Grant, Jablons, & Maas, 1981). The impairment of this ionic pump causes cytotoxic edema and membrane potential alteration, thus interfering also with cell-to-cell communication within the NVU. Furthermore, as said, ATP reduction impairs protein metabolism. This likely indirectly contributes also to autophagy impairment (Mai, Brehm, Auburger, Bereiter-Hahn, & Jendrach, 2019), which might concur to amyloid Aβ accumulation; it is worth noting that NA loss by itself might concur to hamper autophagy (Giorgi et al., 2017). Furthermore, oligoemia-related ATP loss leads to reduced synthesis, among others, of endothelial TJ, in parallel with the loss of the direct promoting effect of NA itself on TJ synthesis (Kalinin et al., 2006). Again, ATP loss might impair also endothelial transcytosis (Mai et al., 2019); also in this case, LC alteration might concomitantly affect directly this process (Harik & McGunigal, 1984; Figure 2 – Panel C).
3. Altogether, the abovementioned phenomena lead to an impairment of BBB permeability which might play a crucial early role in AD pathogenesis, also according to the "two-hit model" by Zlokovic (2011): BBB dysfunction may allow the penetration into the brain of blood-circulating cytotoxic proteins (e.g., thrombin, plasmin), and at the same time it might interfere with the excretion of toxic products from the brain to the bloodstream (Montagne, Zhao, & Zlokovic, 2017). Aβ peptide is shuttled across the vessel walls through transendothelial transport; it is carried from the luminal to the antiluminal side of the ECs after binding to LRP1, whose expression is significantly reduced in AD (Donahue et al., 2006). Even though no specific data on the role of LC on expression and functioning of LRP1 exist, it has been shown that LC stimulation significantly increases BBB permeability to macromolecules by increasing transcytosis (Borges et al., 1994; Harik & McGunigal, 1984; Sarmento et al., 1991, 1994).
4. Kelly et al. recently directly verified such a hypothesis in AD model, as they showed that LC terminal lesion significantly increases the leakage of albumin into brain parenchyma occurring in the Tg344-19 rat model of AD (Kelly et al., 2019).
5. LC lesion induces frank alterations at the level of vessel walls, which further modify BBB permeability and function. In line with this, Kelly et al. clearly showed that Aβ accumulation, up to frank CAA pattern, occurs in Tg344-19 rats undergone LC lesion, much earlier than in littermate rats with an intact LC (Kelly et al., 2019). This *scenario* is supported also by histological and ultramicroscopic observations in the brain of AD patients, showing that cortical CAA and capillary microangiopathy is paralleled by a progressive loss of noradrenergic terminals, even leading Scheibel et al. (Scheibel, 1987; Scheibel, Duong, & Tomiyasu, 1987) to introduce the term "denervating microangiopathy" to emphasize that widespread microvascular pathology may be caused, indeed, by loss of the perivascular neural plexus.
6. Concerning the capillary wall, LC degeneration might significantly affect also PC function and this might further contribute to AD pathogenesis. LC-NA terminals directly contact PC (Paspalas & Papadopoulos, 1996) and induce PC contraction (Peppiatt, Howarth, Mobbs, & Attwell, 2006) through their AR (Asashima et al., 2003; Elfont et al., 1989). Not only their role in neurovascular coupling (Iadecola, 2017) might *per se* be affected by LC degeneration, but it has also been shown that mice bearing constitutive reduced PC number bear an impaired Aβ clearance (Sagare et al., 2013), and thus PC impairment might concur significantly also to Aβ accumulation. In line with this, the study by Kelly et al. (2019) showed that in Tg344-19 rat submitted to LC lesion, in parallel with increased CAA there is a marked increase in wall thickness in distal brain arterioles and they speculated that could be due to PC dysfunction which had been potentiated by LC lesion (Kelly et al., 2019).
7. Neuroinflammation plays a key role in AD pathogenesis and it might be triggered/potentiated by the abovementioned alterations of different components of NVU. For instance, damaged endothelium releases cytokines, and amyloid accumulation causes glial activation (De Luca, Colangelo, Alberghina, & Papa, 2018; Ulrich et al., 2018). Remarkably, one of the best-described mechanisms through which LC potentiates AD pathology in animal models is indeed a potentiation of neuroinflammation (e.g., Giorgi et al., 2019). With this respect, it might be hypothesized that neuroinflammation is the core of a vicious cycle in which LC potentiates NVU damage and altered Aβ clearance, which concurs to activating inflammation, which in turns further potentiates both NVU suffering and AP accumulation in a self-perpetuating/increasing fashion.

As a final aspect to be kept into account regarding NA and AD pathogenesis through NVU impairment, one cannot avoid mentioning GlyS. As already mentioned, GlyS activity and Aβ clearance from the IF varies along the S/W cycle, being higher during sleep and lower in wakefulness (Xie et al., 2013). Xie et al. (2013) have shown that GlyS is strongly regulated by NA, which has inhibitory effects on GlyS, by reducing IS flow and volume (Xie et al., 2013). Furthermore, LC activity variation along the S/W cycle (with high LC activity during wakefulness, and reduction/suppression during sleep) has been even considered pivotal for the GlyS circadian fluctuation above-described (Xie et al., 2013). The latter data are only apparently in contrast with the putative beneficial role of NA activation on Aβ clearance. In fact, proper functioning of GlyS might relay also on the fluctuation of GlyS and NVU/BBB along the S/W cycle; this might get lost in the case of LC degeneration, which might also cause an unbalance of these two amyloid clearance systems, that is, BBB- and GlyS-mediated ones, eventually leading to increased Aβ deposition and AP formation.

To conclude, LC might be key in triggering and promoting the abovementioned chain of events involving the NVU, which concur together with other concomitant factors leading to Tau- and Aβ-related pathology, to the pathogenesis of AD.

8 | CONCLUSIONS

Several experimental studies have shown in the last decades the potential relevant role of LC on the integrity and function of different components of NVU; most of these had been obtained in complex experimental settings and by profiting from a variety of experimental tools, and thus they were often difficult to be related only to LC function. In this review, we tried to put together and discuss critically these studies in a systematic way. The involvement of NVU might reconcile different hypotheses concerning the pathogenesis of AD, and the LC seems to be a particularly appropriate candidate putting in relation with each other all of these factors. Unfortunately, thus far only one study in AD Tg model was designed specifically to directly address the contribution of LC to AD pathogenesis through its effects on NVU (Kelly et al., 2019), while another one assessed the contribution of different AR to the GlyS-mediated Aβ clearance in AD Tg mice (Xie et al., 2013). Therefore, it is desirable that the role of LC will specifically be tested, either by a direct stimulation or a selective lesion of this nucleus, on NVU alterations also in other AD models, both *in vivo* and *in vitro*, and focusing on the effects on different parts/functional components of NVU.

In line with this, recently exciting perspectives come from experimental tools allowing the specific stimulation of target neuronal populations. For instance, that is the case of DREADDs (designer receptors exclusively activated by designer drugs), which are bioengineered receptors that can be expressed in cells via viral vector transfection and through which cellular activity could be precisely modulated (Roth, 2016). Such a technique has been already used in some studies on LC with interesting results (Fortress et al., 2015; Rorabaugh et al., 2017; Vazey & Aston-Jones, 2014).

Moreover, it is worth mentioning that recently, innovative MRI techniques have allowed visualizing in humans, *in vivo*, the integrity of LC (Betts, Kirilina, et al., 2019), and promising PET studies, profiting of new radiotracers specific for NA terminals, might disclose in patients alterations of LC axon terminals (Knudsen et al., 2018). Finally, sophisticated fMRI approaches are already available to unveil the potential alterations of neurovascular coupling in patients (Chen, 2019).

Thus, it is conceivable that in the near future the hypothesis of a link between LC degeneration and NVU impairment in AD pathogenesis might be directly tested by sound translational research approaches. This might further confirm (together with the already existing large amount of data on the role of LC in amyloid-related inflammation) the potential role of brain NA system as a target for innovative disease-modifying therapeutic approaches in AD.

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CONFLICT OF INTEREST

The authors do not have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

F.S.G.: *Conceptualization (equal)*; *Writing – Original Draft Preparation (lead)*; *Writing – Review & Editing (equal)*. F.F.: *Conceptualization (equal)*; *Supervision (lead)*; *Writing – Review & Editing (equal)*. A.G.: *Investigation (lead)*; *Writing – Original Draft Preparation (equal)*; *Visualization (equal)*. S.P.-A.: *Supervision (supporting)*; *Writing – Review & Editing (equal)*. F.L.: *Visualization (equal)*. C.L.B.: *Supervision (supporting)*; *Writing – Review & Editing (equal)*.

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