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The neural basis of functional neuroimaging signal with positron and single-photon emission tomography

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Abstract. Functional imaging techniques such as positron and single-photon emission tomography exploit the relationship between neural activity, energy demand and cerebral blood flow to functionally map the brain. Despite the fact that neurobiological processes are not completely understood, several results have revealed the signals that trigger the metabolic and vascular changes accompanying variations in neural activity. Advances in this field have demonstrated that release of the major excitatory neurotransmitter glutamate initiates diverse signaling processes between neurons, astrocytes and blood perfusion, and that this signaling is crucial for the occurrence of brain imaging signals. Better understanding of the neural sites of energy consumption and the temporal correlation between energy demand, energy consumption and associated cerebrovascular hemodynamics gives novel insight into the potential of these imaging tools in the study of metabolic neurodegenerative disorders.

Keywords. FDG-PET, rCBF-SPECT, brain activity, neurotransmission, neuroenergetics, neuroimaging signal.

Oxidation of glucose is known to provide almost all of the energy needed by neurons to support brain activity. Glucose metabolism is widely distributed throughout brain tissue, with higher rates in the cortex and basal ganglia, two areas of intense neural activity. Glucose metabolism may also vary with time, with rapid increases being detectable in discrete regions upon afferent stimulation. The strong correlation between neural activity and glucose metabolism provides the foundation for a brain imaging method called fluorodeoxy-glucose positron emission tomography (FDG-PET) that maps regional brain activation using glucose analogs [1, 2] (Fig. 1a). Although there is a general consensus that the main source of glucose consumption is glutamate signaling, recent results have changed the traditional view that glucose is consumed exclusively by neurons and that glucose consumption directly reflects neural activity [3]. In this respect, the importance of astrocytes in glutamate-driven glucose metabolism and regulation has been highlighted [4, 5]. Several experiments have shown that this type of glial cell in the central nervous system is closed to glutamatergic synapses and responds to neural activity by consuming more glucose and producing more lactate [6].

In parallel, there is evidence that neurons preferentially oxidize lactate present in the extracellular space rather than glucose to meet their energy demand [7, 8]. Specifically, physiological activation

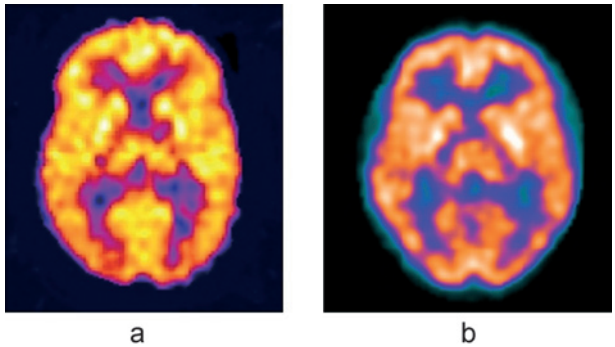

Figure 1. (*a*) FDG-PET scan of normal 45-year-old brain and (*b*) rCBF-SPECT scan of a normal 35-year-old brain.

of a given cerebral area is associated with the release from afferent pathways of glutamate, the principal excitatory neurotransmitter [9, 10]. Stimulation of excitatory glutamatergic neurons may activate AMPA receptors in the postsynaptic terminal of dendritic spine and induces an excitatory postsynaptic potential. Depolarization propagates from dendritic spine to dendrite, where it opens further voltage-gated sodium channels. The rapid increase in Na concentration within the postsynaptic terminal in dendritic spine and dendrites is followed by prompt activation of Na^+/K^+-ATPase. This leads to a rapid increase in energy demand (adenosinetriphosphate, ATP) with subsequent activation of oxidative phosphorylation and reduction in mitochondrial NADH content (the reduced form of nicotinamide adenine dinucleotide) [3, 7]. The recovery of NADH in dendrites is prompt due to rapid stimulation of the tricarboxylic acid (TCA) cycle, fueled mainly by lactate from the extracellular pool. Along with activation of oxidative phosphorylation in mitochondrial neurons, glycolysis is strongly activated in the cytoplasm of astrocytes at glutamatergic synapses, as indicated by the large increase in cytosolic NADH, due to the recycling process of glutamate. Presynaptically released extracellular glutamate is avidly taken up by astrocytes that ensheathe synaptic contacts through high-affinity excitatory amino acid transporters (EAATs) [11]. Thereafter, they are converted in glutamine by glutamine synthetase, which is present in astrocytes but not in neurons, and transported back to neurons for reuse. Glutamate entry entails the cotransport of Na^+ into glia. Excess Na^+ is removed by the Na^+/K^+-ATPase through ATP hydrolysis. Low ATP levels stimulate glial glycolysis. The glucose is delivered from blood flow to the extracellular brain space and astrocytes via astrocytic protrusions called end feet that are in close contact with the blood vessel wall. Glucose is regulated by glutamate release itself. Despite the lack of glutamate receptors in the vasculature and of any direct effect of glutamate on isolated arteries in vitro [12], it has been demonstrated that glutamate actually triggers *in vivo* regional cerebral blood supply as well.

Along with activation of AMPA receptors, glutamate activates NMDA and metabotropic glutamate receptors (mGlu) with subsequent increase in intracellular Ca^+. This triggers the synthesis and release of the potent vasodilator nitric oxide (NO) [13, 14]. Indeed, the involvement of NO in mediating the effect of glutamate is supported by the fact that NO synthase (NOS) is physically anchored to the NMDA receptor by two postsynaptic density proteins, PSD-93 and PSD-95 [15, 16]. Astrocytes may also participate in local perfusion regulation by means of NO and can respond to glutamate activation with rhythmic elevations of intracellular Ca^+ levels that can spread to adjacent astrocytes as waves [17] and exert distant control of microvasculature [18]. In this context, note that NO is not the only enzyme system that produces vasoactive molecules upon glutamate-induced changes in intracellular Ca^{2+}. Phospholipase A_2 releases arachidonic acid (AA) from membrane phospholipids in a Ca^{2+}-dependent fashion. AA is then metabolized via cyclooxygenases (COXs) to a number of vasoactive prostaglandins. Since COXs may be localized in postsynaptic elements of excitatory neurons, where its constitutive expression is coupled to synaptic activity, it has been suggested that vasodilator prostaglandins can couple synaptic activity to cerebral blood flow via glutamate-induced increases in intracellular Ca^{2+} and activity of the phospholipase A_2-COX system [10]. Glucose transport from the blood-brain barrier to the cytosol of brain cells is possible by hexose transporters of the GLUT family, respectively localized on the surface of endothelial cells (GLUT1 55 kDa), neurons (GLUT3) and glial cells (GLUT1 44kDa) [19]. Parallel activation of glucose transport and metabolism is required for glycolitic flux increase in the brain. In contrast with past views, recent studies have shown that the value of the brain glucose concentration implies that glucose transport is rate-limiting for glucose usage and that metabolic activation, however intense, cannot augment flux on its own [20].

To maintain high glycolitic flux in the cytoplasm of astrocytes, NAD^+ must be regenerated via the conversion of pyruvate to lactate through the activity of the enzyme lactate dehydrogenase. Thereafter, the lactate is released into the extracellular space to replenish this space and to sustain the late phase of neuronal activation. Lactate produced by astrocytes is transferred to neurons by monocarboxylate transporters and oxidized in parallel with glucose, thus reducing the amount of glucose used by neurons The overall process has been designated the astrocyte-neuron lactate shuttle hypothesis [3]. The rapid activation of oxidative phosphorylation and lactate oxidation via the TCA cycle in neurons following the glutamatergic transmission is consistent with brain energy studies which have shown that most brain energy is used to power postsynaptic currents and action potentials rather than presynaptic or glial cell activity [10, 21]. Thus, ATP requirements in neurons are fulfilled predominantly via oxidative phosphorylation because this is a more efficient pathway than glycolysis to sustain the high energetic demand. Owing to this difference in efficiency and despite the more important energetic needs of neurons, astrocytes

contribute significantly to glucose consumption and blood supply.

Over the last decade, advances have been made in our understanding of the molecular genetics and pathophysiology of neurodegenerative disorders including Alzheimer's disease (AD), Parkinson's disease (PD) and amyotrophic lateral sclerosis (ASL). Despite the differential clinical features of the various neurodegenerative disorders, the fact that neurons are highly dependent on oxidative energy metabolism has suggested a unified pathogenetic mechanism of neurodegeneration, based on an underlying dysfunction in mitochondrial energy metabolism, oxidative stress and protein mishanding [22]. Particularly, the process of ageing has been considered to be central to all disorders [23, 24]. Cells in the brain are affected by ageing much as cells in other systems. Consequently, cells in the nervous system experience increased amounts of oxidative stress, perturbed energy homeostasis, accumulation of damaged proteins and lesions in their nucleic acids. These molecular alterations during normal ageing are amplified in vulnerable neuronal populations by disease-related processes, which results in their dysfunction and death. Thus, whether an individual develops a neurodegenerative disorder during ageing is determined by genetic and environmental factors that counteract or facilitate fundamental molecular and cellular mechanisms of ageing.

Vulnerable neurons are typically large, with myelinated axons that extend relatively long distances, from one region of the nervous system to another or from the central nervous system to peripheral targets. The reasons that account for the high vulnerability include high energy requirements; large cell surface area, which increases the exposure of the cells to toxic environmental conditions; and reliance on axonal transport. As highlighted in an interesting review [23], this is the case of hippocampal and frontal lobe pyramidal neurons in AD, dopaminergic neurons in the substantia nigra in PD, striatal medium spiny neurons in HD and lower motor neurons in the spinal cord in ASL. Importantly, it has been highlighted that synapses are the most vulnerable regions of neurons. Disease-related stressors may promote activation of biochemical cascades that result in ion dysregulation and energy depletion in the synaptic terminal and neuritis. It has also been suggested that changes that occur in the cellular milieu in which neurons reside, including astrocytes, may also influence the process of neuronal degeneration. The neurotransmitter phenotype has been also shown to be a crucial determinant of selective neuronal impairment. Dopaminergic neurons are most vulnerable in PD, while cholinergic motor neurons and γ-aminobutyric acid-containing striatal neurons are impaired in ASL and HD. Moreover, glutamate has been shown to have an essential role in primary neuronal damage in all neurodegenerative disorders as well in the secondary more widespread but inadequately characterized neuronal loss in other brain regions. In this respect, convergent evidence suggests that patterns of neuronal impairment often are designed to be often domino-like [23]. For example, impairment of neurons in the entorhinal cortex that provide input to the hippocampus degenerate early in the course of AD. The lack of excitatory signal transmission is followed by degeneration of hippocampal neurons and finally of cortical neurons that communicate with hippocampal ones. Similarly, it has been hypothesized that PD is characterized by degeneration of neurons in the medulla oblongata and raphe nucleus and locus ceruleus of the brainstem, which is followed by progressive impairment of nigral neurons, and then in the trans-entorhinal cortex and motor cortex, particularly the pre-supplementary motor area (pre-SMA) [25]. Recently, degeneration in motor neurons in ASL was shown to be progressive from the lower to upper spinal cord and to be followed by impairment in cortical regions of the brain, particularly the frontal cortex and anterior cingulate gyrus [26, 27].

Considering neural information processing, the changes that occur in neurodegenerative disorders and the cellular target of brain imaging radiopharmaceuticals [28, 29], several considerations can be made toward the neural basis of functional brain imaging signal and the potential of emission tomography approaches in the study of human brain disorders. First, glucose consumption (Fig. 1a) and perfusion (Fig. 1b) are regionally regulated by a common determinant, namely glutamate activity and metabolism. Although available data do not yet provide definite answers, they strongly suggest that glutamate could coordinate both the vascular and metabolic responses to neuronal activity that underlie functional imaging signal changes. These effects could involve its receptor-mediated action (ANMPA, NMDA, mGlu) on neuron and astrocytes (metabolic and vascular responses), as well as its transport within the astrocyte (metabolic response). Second, regional changes in glutamate transmission require a fast energy demand which is satisfied by oxidative phosphorylation in neuronal dendrites and glycolysis in astrocytes. As a consequence, based on the known biochemical mechanisms involved in the cell retention of FDG and perfusion radiopharmaceuticals [29], measurements of regional rates of glucose utilization and perfusion with FDG-PET and rCBF-SPECT (regional cerebral brain profusion single-photon emission computed tomography) primarily reflect metabolic activity of

astrocytes that ensheathe synaptic contacts and hemodynamic changes induced by glutamate transmission rather than neuronal activity directly. Furthermore, PET and SPECT techniques require long sampling times to obtain a signal of good quality and so are more likely to detect long-lasting signals – such as the accumulation of both fluorodeoxy glucose in response to glucose uptake and perfusion radioligands in response to hemodynamic changes – rather than early or brief events such as the activation of cellular respiration. Third, although functional brain imaging methods provide only indirect maps of neural activity, the activation of both cell types is strongly correlated, as suggested by the astrocyte-neuron lactate shuttle and glutamate recycle processes [3].

It is noteworthy that astrocyte activity is mainly driven by the increased energy demand at postsynaptic level, as also highlighted by the high proportion of mithochondria found at this site [4, 21]. Metabolic coupling between neurons and glia is consistent with the realization that glia not only support neurons, but these two cell types are highly interdependent for normal nervous system development and function. For example, it has been shown that not only synaptic transmission but also synaptic plasticity require neuron-glia interaction and that glia communicate with neurons during higher brain function, possibly maintaining an appropriate balance of extracellular ions or small molecules to facilitate neural function [11]. Fourth, beside normal neuronal and glial physiology, advances in our understanding of the molecular genetics and pathophysiology of neurodegenerative disorders have clearly shown that degeneration occurs preferentially at the synaptic level and involves dysfunction in mitochondrial energy metabolism, which is mandatory to power postsynaptic currents and action potentials in dendrites. Besides neuronal impairment, it has been shown that astrocyte degeneration is also common in neurodegenerative disorders. Thus, a match exists between the site of neurological dysfunction and the neural basis of functional imaging signal with FDG-PET and rCBF-SPECT. For these reasons, these techniques are nowadays considered the most appropriate approaches to track local energetic transformations in the living human brain [30, 31]. Since functional imaging signals are closely linked to the activity of glutamatergic synapses, any dysfunctioning of the dialog between glutamatergic neurons and astrocytes could lead to altered functional brain images.

Advances in emission tomography techniques and a refined understanding of neural signaling mechanisms in normal and pathological conditions have led to a deeper awareness of the potential of FDG-PET and rCBF-SPECT to investigate the functional anatomy of human brain function (knowing where), to detect metabolic changes at any point of this network during normal and pathological conditions (knowing what), and to characterize the time of appearance of such regional changes (knowing when) [32, 33, 34]. Concerning the study of neurodegenerative diseases, the net changes in regional synaptic activity can be investigated in the primary sites of neuronal degeneration as well as in the secondary widespread brain regions spatially removed from the neurochemical locus of pathology. While measurements at primary sites of degeneration over time can provide insight into the natural history of disease, the study of functional alterations in brain regions distant from the primary locus of pathology can be relevant to study the domino-like effect previously described and the emerging beneficial role of neural compensatory mechanisms outside the primary sites of neurodegeneration. The relationship between brain regions showing a change in neural activity and the clinical status of the patients during scanning can be investigated to reveal the plausible circuits involved in the genesis of cardinal symptoms of neurodegenerative disorders, to drive drug development and to follow the effect of therapies on brain regions with abnormal metabolism.

Along with visual inspection of nuclear brain images, such information can be addressed by using more objective semiquantitative methods based on a voxel-by-voxel analysis [29]. In this case, each voxel within the brain emission volume can be viewed as a cube containing a 3D quantity of glucose or rCBF whose value may vary as a function of the activity of the synapses contained in the voxel (Fig. 2). The information given by the non-invasive functional imaging approaches with PET and SPECT are of crucial importance in basic and clinical neuroscience. For example, results of functional imaging studies in PD have clearly shown that overactivity of basal ganglia outputs in PA does not necessarily lead to generation of symptoms, whereas impairment of the supplementary motor area does. As a consequence, it has been suggested that compensatory mechanisms outside the basal ganglia exist to prevent the appearance of dopaminergic symptoms, even though the basal ganglia are in a parkinsonian-like state. Besides, recent functional imaging studies have led to new insight into the neural network underlying non-dopaminergic symptoms, which have been shown to have a critical role in quality of life in late-stage PD [35]. Although these data do not yet provide definitive answers, they have led to the opinion that restoration of the nigrostriatal dopamine system should not be the ultimate goal of future research [36, 37]. Indeed, starting from the knowledge that disease progression

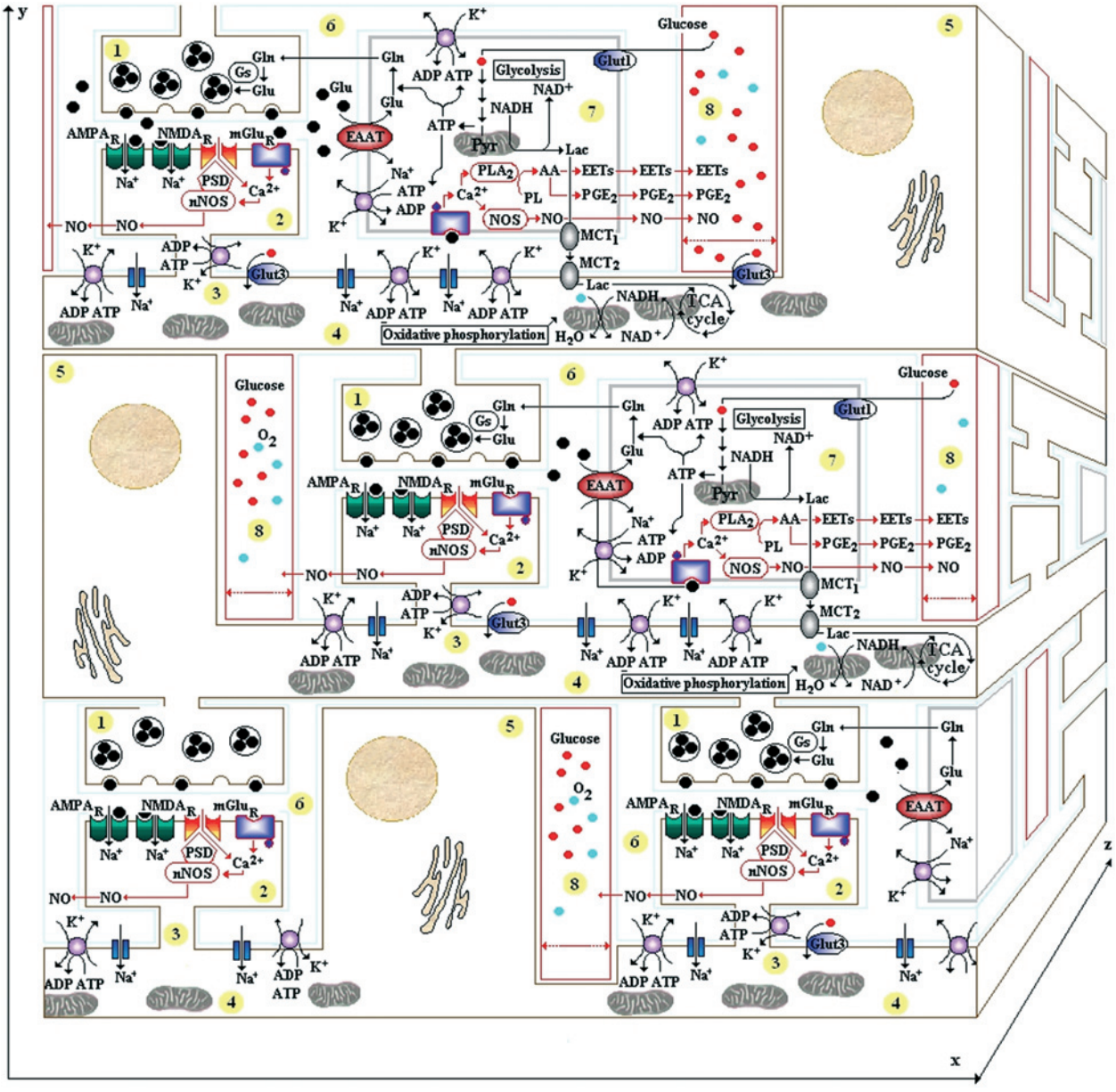

Figure 2. Each voxel of fluorodeoxy-glucose positron emission tomography (FDG-PET) and regional cerebral brain prozusion single-photon emission computed tomography (rCBF-SPECT) images reveals the overall 3D changes in glucose consumption and perfusion as a function of the activity of the synapses contained in the voxel. Both parameters are regionally regulated by a common determinant, namely glutamate activity. The effect of glutamate is mediated by the action of ANMPA, NMDA and mGlu receptors localized on neurons and astrocytes. The regional changes in glutamate transmission require a fast energy demand which is satisfied by an oxidative phosphorylation in neuronal dendrites and glycolysis in astrocytes. As a consequence, based on the known biochemical mechanisms involved in the cell retention of FDG and perfusion radiopharmaceuticals, measurements of regional rates of glucose utilization and perfusion primarily reflects metabolic activity of astrocytes that ensheathe synaptic contacts and hemodynamic changes induced by glutamate transmission to support glucose delivery rather than neuronal activity directly. Nevertheless, a strong correlation exists between neuronal oxidative metabolism and astrocytic glycolysis as highlighted by the neurometabolic coupling in which early oxidative metabolism in neurons is sustained by late activation of the astrocyte-neuron lactate shuttle. This correlation is also sustained by the glutamate recycle processes and by the fact that glutamatergic transmission regulates local rCBF by NMDA and mGlu receptors which are localized on both cell types. Legenda: Red arrows and boxes indicate neurovascular processes, while black arrows and boxes indicate neurometabolic processes. To increase clarity, neuron, astrocyte, capillary and extracellular space are represented in compartmental fashion and highlighted with different colors and numbers: brown = neuron, gray = astrocyte, red = capillary, light blue = extracellular space; (1) = presynaptic neuron, (2) = postsynaptic neuron, (3) = dendritic spine, (4) = dendrite, (5) = neuron, (6) = extracellular space, (7) = astrocyte, (8) = capillary.

remains unaffected despite therapies, several efforts are now devoted to discovering effective treatments that increase neural activity in brain regions that compensate for the appearance of dopaminergic signs (i.e. effectively resulting in an increase of the pre-symptomatic period of PD) and to treat those responsible for the appearance of non-dopaminergic symptoms during the late stage of disease.

Although perfusion and metabolism share a common potential in clinical use, some biological differences exist between these two different activity signals with respect to their spatial spread. Indeed, besides the well-known differences in PET and SPECT scanners and kinetics underlying the distribution of flow and metabolism tracers in the brain [28, 29], a biological smoothing effect has also been suggested that may contribute to the higher spatial resolution of PET versus SPECT [38]. More specifically, it can be assumed that local energetic impairment due to a degenerative disorder should consequently decrease glucose metabolism and blood perfusion. While glutamate activity and glucose regulation are spatially restricted by an efficient glutamate uptake system and diffusion [39], low molecular weight and hydrophobic properties makes NO a molecule which rapidly diffuses and permeates cell membranes [40]. It has been estimated that the physiological sphere of influence of a single source producing NO has a diameter of 200–600 μm, which corresponds to a volume of brain containing 2 million synapses. It has also been shown that tissue changes due to neurodegenerative disorders may decrease the number of specific target molecules for NO, resulting in a net increase of the path of NO in the affected tissue. Consequently, NO from surrounding regions characterized by a normal NO level might permeate into regions with low NO due to low glutamate release. The high diffusion of NO from brain regions with normal NO to regions with low NO might obscure the reduction in perfusion in comparison with the metabolic reduction. Moreover, NO delivered from astrocytes may also contribute to smoothing the sharpness of perfusion defects [34].

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