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# Dysregulation of Nitric Oxide Signaling in Microglia: Multiple Points of Functional Convergence in the Complex Pathophysiology of Alzheimer Disease

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Study Design A

Data Collection B

Statistical Analysis C

Data Interpretation D

Manuscript Preparation E

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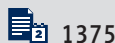
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Current critical thinking has displaced the elaborated beta amyloid theory as the underlying unitary mechanism of Alzheimer disease (AD) in favor of concerted, long-term disruption or dysregulation of broad-based physiological processes. We present a critical discussion in which a chronic state of systemic proinflammation sustained over the course of several decades and engendered by ongoing metabolic or autoimmune disease is predicted to promote severe disruptions of central neurological processes. Specifically, long-term functional rundown of microglial-mediated phagocytic activity in concert with aberrant expression and cellular deposition of beta amyloid and tau protein facilitates formation of senile plaques and neurofibrillary tangles. Within this functional context, we hypothesize that early initiation events in the pathophysiology of AD may operationally involve a convergence of dysregulated peripheral and central constitutive nitric oxide signaling pathways resulting from a chronic state of systemic proinflammation and leading to severely dysfunctional "hyper-activated" microglia.

**MeSH Keywords:** **Alzheimer Disease • Amyloid beta-Peptides • Microglia • Nitric Oxide • tau Proteins • Ubiquitins**

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## Background

The worldwide prevalence and intractability of severe neurological manifestations of Alzheimer disease (AD) across aging populations were highlighted in a recent series of related articles in *Scientific American* (volume 322, issue 5, May 2020). The lead article by Kenneth Kosik presented a selectively focused discussion that essentially minimizes the primacy of beta amyloid and its deposition in extracellular senile plaques as the “gold standard” causative mechanism driving the complex pathophysiology of AD (The Way Forward: 30–36). This provocative contention is supported by the observed lack of significant efficacy of several high-affinity monoclonal antibody therapeutic agents to selectively inhibit or disrupt beta amyloid-mediated plaque formation with subsequent attenuation of cognitive decline. Kosik also reported that concerted, long-term disruption or dysregulation of broad-based physiological processes such as intracellular protein degradation by the ubiquitin-proteasome system and/or lysosome-mediated autophagy underlie the pathophysiological expression of both beta amyloid deposition and tau protein aggregation within neurofibrillary tangles.

Of potentially greater importance, phenotypic expression and concerted functional activities of previously identified high-risk genetic polymorphisms, notably *APOE4*, *TREM2*, and *BIN1*, have been critically linked to dysregulation of requisite phagocytic activities of microglia, the resident immune cells of the central nervous system (CNS) [1–6]. Accordingly, long-term rundown of microglial-mediated phagocytic activity in combination with aberrant expression and cellular deposition of beta amyloid and tau protein may represent a multifaceted underlying mechanism that facilitates formation of senile plaques and neurofibrillary tangles. From a global perspective, a chronic state of systemic proinflammation sustained over the course of several decades and engendered by ongoing metabolic disease such as type 2 diabetes or autoimmune dysregulation is predicted to promote severe disruptions of central neurological processes, as reviewed by Esch and Stefano [7] and Morris et al. [8]. It is then reasonable to hypothesize that the concurrent cellular and behavioral manifestations of AD (i.e., neuronal degeneration and cognitive dementias) are critically linked to chronically “hyperactivated” microglia within discrete CNS areas [2,9].

## Nitric Oxide Dysregulation and Proinflammation

Previous work from our group has focused on the biological importance of constitutively expressed nitric oxide (NO) as a key regulatory molecule in the mediation of innate immunological responses in diverse cell types and in proinflammatory-associated adaptive immunological challenges, as reviewed

in Stefano et al. [10]. NO has been empirically established as a key signaling molecule in multiple regulatory pathways underlying vascular, metabolic, immune, and neurological function across animal phyla [10–12]. Interestingly, prior work from our group has demonstrated that loss of NO-mediated inhibitory tone is functionally linked to inflammatory damage to AD brain microvasculature, as reflected by immunocyte adherence, endothelial shape changes, and loss of integrity of gap junctions in juxtaposed cells [13]. Furthermore, the resultant diminution of essential blood-brain barrier function has been well documented in AD and has been proposed as a significant causative factor in the formation of chronically hyperactivated microglia, as reviewed by Morris et al. [8]. Accordingly, we hypothesize that early initiation events in the pathophysiology of AD may operationally involve a convergence of dysregulated peripheral and central constitutive NO signaling pathways resulting from a chronic state of systemic proinflammation and leading to severely dysfunctional microglia.

In support of the contentions stated above, preclinical studies have observed that genetic ablation of the gene encoding the constitutively expressed endothelial isoform of NO synthase (eNOS) is associated with increased expression of amyloid precursor protein and beta amyloid peptides A $\beta$ 1–40 and A $\beta$ 1–42, as reviewed by Katusic and Austin [14]. Additionally, enhanced production and deposition of A $\beta$  peptides were observed in the cerebral microvasculature and brain tissue of eNOS-deficient mice. In these same animals, genetic ablation of eNOS was associated with activated microglia, indicating the establishment of a proinflammatory state within CNS structures. Finally, eNOS-deficient mice exhibited impaired cognitive performance in established behavioral paradigms, strongly suggesting that selective loss of regulatory NO signaling results in profoundly debilitating downstream effects on coupled vascular and neuronal functions [14]. Interestingly, prior studies from our group employing excised invertebrate ganglia in an *ex vivo* model of neuronal injury demonstrated time-dependent egress of microglial cells into the culture media [15]. Released microglia were observed to undergo conformational changes ranging from stellate to rounded to partially amoeboid morphologies, and comparable to similar morphological changes observed in activated invertebrate immunocytes [15–18]. Pharmacologically mediated restoration of low concentrations of NO, simulating ongoing regulatory activities mediated by constitutive NOS, was observed to coordinately decrease the number of microglial cells emerging from the excised ganglia and the degree of their transformation to the “active” amoeboid form [16–18]. In sum, constitutively expressed NO appears to represent a key regulatory/inhibitory factor in a translationally valid invertebrate model of microglial activation.

More recently, in a preclinical cell culture model of microglial activation, lipopolysaccharide stimulation was employed to

simulate a state of chronic inflammation via expression of the inducible isoform of NOS [1]. Activated microglia were observed to possess enhanced phagocytic activity against cellular debris produced by apoptotic neuronal cell lines. Additionally, activated microglia were observed to inhibit neurite outgrowth from a co-cultured human neuronal cell line. Importantly, pharmacological blockade of the inducible isoform of NOS catalyzed NO production and release of high concentrations of NO from lipopolysaccharide-stimulated microglia completely revised the inhibitory effects on neurite outgrowth in concert with reductions in phagocytic activity. These combined data suggest that dysregulation of normative NO signaling in combination with canonical oxidative effects of inducible NO may provide a permissive cellular environment for proinflammatory-mediated phagocytic activities by hyperactivated microglia to remove cellular debris arising from neutralized invading pathogens, or in the case of AD, ectopic deposition of beta amyloid peptides and/or aggregated tau in neuronal tissues and surrounding microvasculature [10,13,19,20]. In AD, long-term pathophysiological rundown of essential microglial functions is associated with chronic, peripherally mediated, proinflammatory hyperstimulation in concert with metabolic insufficiencies due to compromised mitochondrial bioenergetics (as reviewed by Morris et al. [8]). Within this same functional context, we have previously proposed that health-related enhancements of whole-body metabolic advantage may be achieved by optimization of synchronous regulation of mitochondrial oxygen utilization by intramitochondrial NO production and release [21]. Accordingly, loss of NO-mediated regulation of mitochondrial oxygen consumption and ATP production may be a significant contributing factor to the pathophysiology of late-onset AD via loss of metabolic integrity of chronically activated microglia.

As discussed, a chronic state of systemic proinflammation sustained over the course of several decades is proposed to represent a highly significant permissive factor in the etiology of late-onset AD. Within this functional context, long-term functional rundown of chronically hyperactivated microglia within discrete CNS areas appears to represent a key causative factor underlying the hallmark cellular and behavioral manifestations of AD (i.e., neuronal degeneration and cognitive dementias) [2,9]. Furthermore, we provide compelling evidence in support of the critical, multifaceted regulatory role of

constitutive NO signaling in innate and adaptive immunological processes mediated by microglia. Accordingly, dysregulation of normative NO signaling in combination with canonical oxidative effects of inducible NO is proposed to represent a permissive cellular environment for proinflammatory-mediated phagocytic activities by hyperactivated microglia to remove ectopic deposition of beta amyloid peptides and/or aggregated tau. Importantly, highly selective diagnostic indicators for the occurrence and severity of early-stage AD are starting to emerge that include quantification of circulating plasma concentrations of phospho-tau217 [22]. The development of high-resolution diagnostic/prognostic tests would justify and facilitate earlier therapeutic intervention. We note that this would be very advantageous for a strategy involving constitutive NO release. Within this functional context, age-associated rundown of essential phagocytic and innate immunological functions in compromised microglia is associated with chronic, peripherally mediated, proinflammatory hyperstimulation in concert with metabolic insufficiencies, presumably due to severe reductions in mitochondrial bioenergetics.

## Conclusions

We hypothesize that long-term maintenance of constitutive NO signaling via combined pharmaceutical, dietary, or complex behavioral interventions may partially provide preemptive protection against deleterious physiological conditions brought about by a chronic state of systemic proinflammation. These restorative interventions may be directed to promote whole-body metabolic advantage via systemic downregulation of excitatory, energy-consuming cellular processes with resultant enhancements of mitochondrial bioenergetics in multiple organ systems [21,23]. In sum, preemptive enhancement of whole-body NO tone may represent a novel approach to optimize human health by incorporating behavioral, physical, and pharmaceutical approaches that effectively target and address slowly developing physiological challenges functionally associated with the etiology of late-onset AD.

## Conflict of interest

None.

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