Journal Club

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Training Microglia to Resist Alzheimer's Disease

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Review of Xu et al.

Alzheimer's disease (AD) is a progressive neurodegenerative type of dementia with no effective treatments. In the search for ways to manage AD, nonpharmacological interventions, focused on patient lifestyle, are steadily gaining ground. For instance, recent evidence indicates that cognitive enrichment may prevent and slow dementia. A meta-analysis (Sajeev et al., 2016) compiling results from 12 studies involving nearly 14,000 individuals concluded that late-life cognitive activity is associated with lower risks of the development of AD. Studies using animals show that environmental enrichment—housing conditions that stimulate physical activity, cognitive exercise, and social interaction—improves cognitive functions in ways that may be beneficial in AD therapy. Environmental enrichment boosts neuronal plasticity and induces adult neurogenesis, while reducing neuroinflammation and neurodegeneration (Fischer, 2016), and in animal models of AD, environmental enrichment has been shown to ameliorate cognitive phenotypes (Jankowsky et al., 2005; Blázquez et al., 2014). However, causal connections are often difficult to establish, and the cellular and molecular mechanisms underlying the benefits of cognitive enrichment in AD are still poorly understood.

Accumulation of oligomeric amyloid-β peptide (oAβ) in the brain is thought to trigger other pathological events leading to cognitive decline in AD. In hippocampal neurons, oAβ attacks synapses, altering receptor composition, impairing plasticity, and eventually causing synapse loss. In parallel, oAβs activate microglia, leading to sustained secretion of neurotoxic cytokines that promote neuroinflammation (Ferreira et al., 2015).

Whether and how environmental enrichment interferes with oAβ toxicity in the brain is a key question in understanding the protection that cognitive enrichment confers against AD. A recent study (Li et al., 2013) showed that long-term exposure of mice to environmental enrichment preserved synapses of hippocampal neurons from an oAβ insult, preventing the impairment of long-term potentiation, an electrophysiological correlate of memory. These findings provided an underlying mechanism for environmental enrichment protection in mice, which can reasonably be extrapolated to explain the benefits of cognitive enrichment in AD. However, AD is a multifactorial disease comprising multiple cellular events, and other factors that may be involved in cognitive preservation by environmental enrichment must be considered. An important unexplored issue is the impact of environmental enrichment on the innate immune system of the brain. Specifically, does environmental enrichment modulate microglial responses to oAβ, and if so, how?

In a study recently published in *The Journal of Neuroscience*, Xu et al. (2016) analyzed the impact of long-term environmental enrichment on the microglial response to oAβ in the dentate gyrus (DG) of the hippocampus of adult wild-type mice. First, the authors showed that mice housed under environmental enrichment for $\geq$4 weeks displayed increased microglial density in the DG (Xu et al. (2016), their Fig. 1). Moreover, automated morphological analysis showed that environmental enrichment increased microglial branching, an indicator of altered microglial function (Xu et al. (2016), their Fig. 3).

To evaluate the ability of environmental enrichment to protect against the impact of oAβ on microglia, naturally secreted oAβ from CHO cells stably expressing human APP were intracerebroventricularly injected into mouse brains. Oligomer injections induced microglial activation in the hippocampus of mice housed under standard conditions, as depicted by morphological alterations and increased expression of the inflammatory activity marker CD68. Remarkably, environmental enrichment robustly prevented oAβ-induced microglial activation (Xu et al. (2016), their Fig. 5).

Received Oct. 28, 2016; revised Dec. 6, 2016; accepted Dec. 12, 2016.

M.N.N.V. is a Postdoctoral Fellow from the Fundação de Amparo à Pesquisa do Rio de Janeiro, and D.B. is a Postdoctoral Fellow from the Conselho de Aperfeiçoamento de Pessoal de Nível Superior. We thank Dr. Luis E. Santos and Professor Sergio T. Ferreira of the Federal University of Rio de Janeiro for their input and critical review of the manuscript.

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DOI:10.1523/JNEUROSCI.3345-16.2017

 0270-6474/17/370477-03$15.00/0

Next, the authors explored the impact of oAβ on the microglial expression profile of inflammatory factors by using the NanoString mRNA analysis. Importantly, the authors used flow cytometry sorting to purify microglia before gene expression analysis, thereby avoiding the confounding effects of contaminating nonmicroglial cells such as peripheral macrophages. Exposure to oAβ changed the expression levels of several inflammatory genes, most of which are implicated in cytokine or JNK signaling. Importantly, environmental enrichment prevented such changes for most genes. Among the genes altered by oAβ, TNF-α, Ccl3, and Ccl4 were the most consistently rescued by environmental enrichment. Induction of TNF-α, Ccl3, and Ccl4 expression by oAβ and prevention of this induction by environmental enrichment were confirmed at the protein level using *in vivo* microdialysis to quantify the cytokines in the brain interstitial fluid (Xu et al. (2016), their Fig. 9). Finally, the rescuing of microglia from an inflammatory phenotype by environmental enrichment was confirmed using oAβ obtained from AD brain extracts (Xu et al. (2016), their Figs. 10, 11).

Strong conclusions can be drawn from these results. The animal model used—wild-type mice challenged with intracerebroventricular injections of oAβ—exclude the confounding effects of APP and presenilin mutations present in transgenic AD models, allowing the direct evaluation of the protective impact of environmental enrichment against oAβ toxicity. This is particularly relevant for sporadic AD, which accounts for >95% of the cases, and in which oAβ accumulates in the brain in the absence of deterministic genetic mutations. This experimental design is more likely than transgenic models to offer a reliable prediction of the microglial reaction to accumulating oAβ in the brains of people with cognitively rich lifestyles with sporadic AD. Additionally, oAβ preparations obtained from cells stably expressing human APP or from extracts of an AD brain were extensively characterized in this and previous studies from the same group to certify that the Aβ species used are indeed pathologically relevant. This is particularly important given the metastable nature of such species and the fact that Aβ biological activity is highly dependent on its aggregation state (Rahimi et al., 2008).

Chronic microglia-mediated neuroinflammation is an important feature of AD physiopathology. Evidence for microglial activation has been found in transgenic AD mice and human AD brains (Solito and Sastre, 2012). Abnormal microglial activation has been hypothesized to impair cognitive function in AD through multiple mechanisms, including sustained secretion of neurotoxic cytokines and synaptic pruning (Hong et al., 2016). Available data also suggest that oAβ directly interacts with microglia to produce neuroinflammation (Ledo et al., 2013), further suggesting that blocking activation of microglia by oAβ could be an effective therapeutic approach to reduce neuroinflammation in AD.

Microglia are complex, dynamic cells that can have supportive or detrimental effects on neurons depending on their activation phenotype and secreted factors (Hanisch and Kettenmann, 2007). Xu et al. (2016) provided compelling evidence that environmental enrichment shifts microglia toward a more benign phenotype, thus suppressing a harmful inflammatory response to oAβ. This finding expands the horizon in the study of cognitive enrichment as a therapeutic approach to AD, highlighting a largely unexplored feature of environmental enrichment as an immunomodulator of the CNS. The fact that microglia-mediated neuroinflammation is not an exclusive feature of AD but commonly occurs in many neurological diseases (Schwartz and Deczkowska, 2016) suggests that environmental enrichment might be beneficial in a wide spectrum of brain disorders.

Another important contribution of the work by Xu et al. (2016) are the robust data pinpointing TNF-α and the related chemokines Ccl3 and Ccl4 (Glabinski et al., 2003) as undisputed targets upregulated by oAβ in hippocampal microglia, and the evidence for the rescuing of such proinflammatory signaling by environmental enrichment training. This is particularly relevant in face of recent evidence implicating TNF-α as a key player in important aspects of AD physiopathology. Specifically, TNF-α has been shown to be involved in synaptotoxicity and cognitive decline caused by oAβ (Bomfim et al., 2012; Lourenco et al., 2013; Kim et al., 2016). Activation of the TNF-α pathway leads to defective insulin signaling in hippocampal neurons through the inhibition of IRS-1—a major effector of insulin signaling—in a mechanism mediated by protein kinase R and involving the stress kinases JNK and IKK (Bomfim et al., 2012) and the endoplasmic reticulum stress mediator eIF2α (Lourenco et al., 2013). TNF-α also induces peripheral insulin resistance in diabetes, and it has been proposed that defective neuronal insulin signaling provoked by TNF-α may underlie the increased predisposition for the development of AD in diabetic patients (De Felice and Ferreira, 2014). Activated microglia are the main source for TNF-α in the brain, and the finding by Xu et al. (2016) that environmental enrichment prevents oAβ-mediated induction of TNF-α secretion by microglia in the hippocampus suggests that cognitive enrichment may provide protection against neuronal insulin resistance and cellular stress responses, mechanisms that may connect AD and diabetes. Therefore, environmental enrichment could represent a particularly important therapeutic approach to prevent the development of AD in diabetic patients.

In addition, recent studies (Ledo et al., 2013) link aberrant TNF-α signaling induced by oAβ to depression, one of the most common neuropsychiatric symptoms associated with AD. The injection of oAβ into mouse brains induces depressive-like behavior through a mechanism involving microglial activation, TNF-α upregulation, and decreased levels of serotonin in cognitive areas, such as the hippocampus and prefrontal cortex. Anti-inflammatory agents infliximab and minocycline, as well as the depletion of microglia, counteracted serotonin downregulation and depressive behavior caused by oAβ (Ledo et al., 2016). In line with recent findings that cytokine modulators such as infliximab ameliorate depressive symptoms in chronically inflamed subjects (Kappelmann et al., 2016) and to the recent link connecting microglial dysfunction and depression in AD (Santos et al., 2016), environmental enrichment may serve as a therapeutic approach to target AD-linked depression.

A fundamental question that remains to be addressed concerns the physiological and molecular mechanisms by which environmental enrichment modulates microglial function. A previous study from the same group showed that environmental enrichment appears to protect neurons against oAβ via activation of β2-adrenergic receptors (β2ARs) and cAMP/PKA signaling, and the protective effects can be mimicked by the oral administration of a β2AR agonist (Li et al., 2013). Xu et al. (2016) speculate that the same pathway may be involved in microglial responses to environmental enrichment, as it was reported that β2AR is expressed in microglia, where it modulates proinflammatory responses and the dynamics of microglial processes. If this is proven to be correct, the β2AR could be a promising pharmacological target for protecting AD brains against oAβ by preventing both synaptotoxicity and microglial dysfunction. Unraveling the novel molecular mechanisms underlying CNS immuno-

modulation by environmental enrichment may provide additional new pharmacological targets for fighting AD and other conditions associated with neuroinflammation, including depression.

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