CHAPTER TWO

Neuroinflammation in organophosphate-induced neurotoxicity

Michelle Guignet, Pamela J. Lein*
Department of Molecular Biosciences, University of California-Davis, Davis, CA, United States
*Corresponding author: e-mail address: pjlein@ucdavis.edu

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1. Introduction

The term "organophosphate" (OP) refers to a group of synthetic organic compounds that have in common a pentavalent phosphorus joined to sulfur or oxygen via a covalent double bond (Costa, 2018). OP compounds were first developed in the early 20th century as insecticides. In the 1930s, it was discovered that the principal molecular mechanism of their insecticidal activity was inhibition of acetylcholinesterase (AChE), an enzyme that is conserved across species, including humans. This discovery unfortunately led to the development during World War II of potent OP

Advances in Neurotoxicology, Volume 3
ISSN 2468-7480
https://doi.org/10.1016/bs.ant.2018.10.003

nerve agents with high human toxicity, including tabun, sarin, cyclosarin, soman, VR, and VX (Costa, 2018; Pereira et al., 2014). Since World War II, hundreds of OP compounds have been developed and marketed as insecticides, fire retardants, plasticizers, and fuel additives, but their most predominant use has been as insecticides (Costa, 2018; Voorhees et al., 2016). By the 1970s, OPs largely replaced organochlorine pesticides because they were equally efficacious, but significantly less persistent in the environment, and by the year 2000, >70% of all insecticides used in the United States were OPs (Costa, 2018; Voorhees et al., 2016). However, over the past decade, residential uses of OP pesticides have been largely phased out and commercial applications of OPs are being increasingly restricted in the United States and many European countries due to increasing concerns of adverse public health impacts (Rohlman et al., 2011; Voorhees et al., 2016). Despite the decline in their use, OP insecticides remain among the most commonly used group of insecticides worldwide, with particularly heavy use in developing countries because of their lower cost relative to newer insecticides (Costa, 2018; Voorhees et al., 2016). As a result of their continued widespread use as insecticides, OPs also remain among the most commonly detected anthropogenic contaminants in the global environment, including human tissues (Barr et al., 2011; Clune et al., 2012; Voorhees et al., 2016).

OPs are primarily neurotoxicants that poison insects by inhibiting AChE, an enzyme that catalyzes the hydrolysis of the neurotransmitter acetylcholine to terminate cholinergic neurotransmission. The phosphate form of OPs, which depending on the OP may be the parent compound or an active metabolite, inhibits AChE by phosphorylating the serine residue in the catalytic triad of the enzyme's active site (Costa, 2006; Eddleston et al., 2008). Inhibition of AChE increases the amount and residence time of acetylcholine at nicotinic and muscarinic cholinergic receptors, resulting in cholinergic overstimulation of target tissues, which immobilizes the insect. However, with few exceptions, OPs are not highly specific for targeted insect species, and mammals, including humans, are susceptible to their neurotoxic effects (Costa, 2018; Rohlman et al., 2011; Voorhees et al., 2016). Acute OP intoxication is estimated to cause >3 million life-threatening human poisonings each year, with approximately 1 million of these cases attributed to occupational exposures, and over 250,000 deaths as a result of self-poisoning, which represents approximately about one-third of the world's suicide cases (Gunnell et al., 2007; Mew et al., 2017; Pereira et al., 2014). In humans, acute OP poisoning causes both peripheral and

central cholinergic effects that collectively define the clinical toxidrome known as cholinergic crisis (Eddleston et al., 2008; Hulse et al., 2014). This toxidrome is characterized by parasympathomimetic symptoms, muscle fasciculation followed by flaccid paralysis, centrally-mediated seizures that can quickly progress to *status epilepticus* (SE), and respiratory failure. Cholinergic crisis is frequently fatal unless rapidly treated with atropine to block muscarinic receptors, oxime to reactivate AChE, and benzodiazepines to reduce seizure activity (Bird et al., 2003; Eddleston et al., 2008). However, clinical trials have revealed no added benefit of using oximes to treat poisoning by various OP insecticides, despite the fact that oxime treatment effectively reactivated blood AChE (Buckley et al., 2011). Even with treatment, survivors of cholinergic crisis often face significant long-term morbidity, including cognitive dysfunction, affective disorders, and spontaneous recurrent seizures (also known as acquired epilepsy) that typically manifest well after cholinesterase activity has recovered to pre-exposure levels (Chen, 2012; De Araujo Furtado et al., 2012; Pereira et al., 2014). Preclinical studies have replicated the effects observed in acutely intoxicated humans, including cholinergic signs, SE, delayed and persistent cognitive dysfunction (Deshpande et al., 2014; Filliat et al., 1999, 2007; Flannery et al., 2016; Myhrer et al., 2005; Raffaele et al., 1987), anxiety-like behavior (Coubard et al., 2008; Prager et al., 2014a; Wright et al., 2010), and spontaneous recurrent seizures (De Araujo Furtado et al., 2010; Shrot et al., 2014). As in humans, long-term effects typically manifest after recovery of cholinesterase activity. Thus, the clinical and preclinical data suggest that while acute, profound AChE inhibition mediates the acute neurotoxic effects of OPs, including seizure activity (Mcdonough and Shih, 1997), it seems likely that the long-term effects of acute OP intoxication are mediated by mechanisms other than or in addition to AChE inhibition.

Repeated low-level OP exposures that do not elicit symptoms of cholinergic crisis are also associated with neurotoxic outcomes in humans, including cognitive deficits and neuropsychiatric conditions such as depression, anxiety, and suicidal tendencies (reviewed in Voorhees et al., 2016). Recent epidemiologic studies suggest that repeated low-level OP exposures may also increase individual risk for neurodevelopmental disorders (Munoz-Quezada et al., 2013; Sagiv et al., 2018a,b) and neurodegenerative disease (Baltazar et al., 2014; Sanchez-Santed et al., 2016). These associations have been challenged by some due to inconsistent outcomes and a lack of epidemiologic evidence demonstrating a dose-response relationship of the neurobehavioral impacts from repeated low-level OP exposures

(Meyer-Baron et al., 2015; Rohlman et al., 2011). However, recent evaluations of the published literature have concluded that the weight-of-evidence supports an association of subclinical occupational (Meyer-Baron et al., 2015; Rohlman et al., 2011) and early-life (Burke et al., 2017) OP exposures with negative impacts on human neurobehavior. Interestingly, these evaluations also concluded that the epidemiologic data do not support an association between OP effects on blood cholinesterase activity and neurobehavioral outcomes (Burke et al., 2017; Meyer-Baron et al., 2015; Rohlman et al., 2011), suggesting there may be non-cholinergic mechanisms of neurotoxicity following repeated low-level OP exposures. A review of the preclinical literature generally supports this hypothesis (Burke et al., 2017). While the most significant and prolonged motor effects are observed following OP exposures that markedly inhibit brain AChE activity, cognitive deficits are not as clearly correlated with AChE inhibition (Bushnell and Moser, 2006). The hypothesis that OP neurotoxicity is not mediated entirely by cholinergic mechanisms is further supported by observations that different OPs elicit different profiles of neurotoxicity despite causing comparable changes in AChE activity and other cholinergic markers (Bushnell and Moser, 2006; Costa, 2006; Pope, 1999). Additionally, AChE knockout mice exhibit symptoms of neurotoxicity comparable to those observed in wildtype mice following OP exposure (Duysen et al., 2001).

Collectively, clinical, epidemiologic, and experimental evidence suggest that mechanisms in addition to or other than AChE inhibition mediate OP neurotoxicity associated with either acute poisoning or repeated low-level exposures (Costa, 2006; Naughton and Terry Jr., 2018; Pope, 1999). This observation has significant implications for the development of effective medical countermeasures for OP poisoning, interventional strategies for preventing OP neurotoxicity, and the use of AChE inhibition as a predictive or diagnostic biomarker of OP-induced neurotoxicity. Various targets and mechanisms other than AChE inhibition have been proposed to mediate OP neurotoxicity, including neuroinflammation (Burke et al., 2017; Costa, 2018; Naughton and Terry, 2018; Pereira et al., 2014). Experimental evidence demonstrates that OPs trigger neuroinflammatory responses under a broad range of exposure paradigms that vary with respect to the type of OP, level of exposure, and exposure duration. There is great interest in confirming a mechanistic link between neuroinflammation and OP neurotoxicity because of the existence of quantitative biomarkers of inflammation that correlate well with neurobehavioral deficits observed in neurodevelopmental disorders and neurodegenerative disease (Chahine et al., 2014; Dziedzic, 2006; Heneka et al., 2015) and the availability of

anti-inflammatory therapeutics approved for use in humans. The remainder of this chapter will provide a brief overview of neuroinflammation, a summary of experimental evidence implicating neuroinflammation as a mechanism of OP neurotoxicity, and a discussion of critical data gaps that need to be addressed to better understand the diagnostic and therapeutic implications of OP-induced neuroinflammation.

2. Overview of neuroinflammation

Inflammation is a cardinal host defense response to pathogen invasion, irritants (including toxic chemicals), and physical trauma. Initially defined in the context of clinical symptoms (pain, redness, heat, swelling), the term "inflammation" is currently used to refer to a complex cascade of cellular processes involving upregulation of proinflammatory cytokines and chemokines, macrophage activation, and leukocyte infiltration of the affected tissue. The initial proinflammatory response functions to eliminate the offending stimulus, and this is typically followed by a resolution phase in which the same stimuli promote different cellular changes to facilitate repair and healing. The inflammatory response can be acute or chronic, and can involve cells and molecules of the innate and/or the adaptive immune system. The term "neuroinflammation" refers specifically to an immune-mediated, glial cell-propagated response organized within the nervous system in response to brain injury or systemic inflammation (Kraft and Harry, 2011; Viviani et al., 2014). Neuroinflammation is critical for neuroprotection and neurorepair following injury to the brain (Konishi and Kiyama, 2018; Kraft and Harry, 2011); however, when dysregulated, neuroinflammation can compromise the function of neural circuits or even cause neuronal cell death, which contribute to the initiation, progression and/or exacerbation of neurologic disease (Herman and Pasinetti, 2018; Shi and Holtzman, 2018). The balance between beneficial and harmful effects of neuroinflammation is determined by the context, magnitude, duration, and timing relative to the primary stimulus of the neuroinflammatory response (Disabato et al., 2016). Whether initiated directly in the nervous system or indirectly in response to a peripheral stimulus, neuroinflammatory responses are orchestrated by multiple resident cell types in the nervous system that signal to each other through the production of second messengers, including cytokines, chemokines, and reactive oxygen and nitrogen species (ROS and RNS, respectively) (reviewed in Disabato et al., 2016; Kraft and Harry, 2011; also see Table 1; Fig. 1).

Table 1 Biomarkers of neuroinflammation.

Inflammatory element	Role in inflammation	Experimental application
Soluble biomarkers		
IL-1α, IL-1β, TNF-α, IL-6, IFN-γ	Pro-inflammatory cytokines produced and secreted by injured neurons and activated immune cells that contributes to the increased inflammatory tone	Secreted biomarkers found in both serum and brain tissue. Expression measured at the mRNA level by qPCR and at the protein level by ELISA or multiplex immunoassays (i.e., Luminex™)
IL-4, IL-10	Anti-inflammatory cytokines secreted by immune cells to dampen inflammation	
CXCL1, MIP-1α, GRO/KC	Chemokines secreted by activated macrophages that recruit neutrophils	
ICAM, VCAM, Selectin	Cell adhesion molecules upregulated on the surface of immune cells in response to cytokine stimulation	
TREM1	Cell surface receptor on myeloid cells that when activated triggers downstream immune response	
PGE2	Bioactive lipid product of arachidonic acid metabolism implicated in inflammatory responses	
SOCS3	Cytokine-inducible negative regulator of cytokine signaling	
Cell surface biomarkers		
Iba-1; IB4 (ILB4), Cd11b, OX-42	Microglia and macrophage specific cell surface markers	Spatiotemporal expression assessed by immunohistochemistry (also enables analysis of cell morphology); expression levels quantified by qPCR or immunoblotting
MAC-1	Complement receptor expressed on neutrophils, natural killer cells and macrophages	
CD68	Lysosomal marker upregulated in phagocytically active immune cells	
GFAP	Glial fibrillary acidic protein, cell surface marker for astrocytes	
In vivo imaging biomarkers		
TSPO	Outer mitochondrial membrane protein whose expression upregulated in activated microglial cells and astrocytes	*In vivo* PET imaging biomarker of neuroinflammation

Microglia are the innate immune cells of the central nervous system (CNS) and are typically the first responders to CNS injury or inflammatory stimuli. Under physiologic conditions, microglia are thought to regulate synaptic activity and to provide immune surveillance (Mosser et al., 2017). Expression of cell-surface purinergic receptors, phosphatidylserine, and toll-like receptors (TLRs) enables microglia to recognize damage associated molecular patterns (DAMPs) and other molecular signals released by injured cells, such as ATP (Saijo and Glass, 2011). Microglia respond to these molecular signals by morphologically transforming from a cell with a small soma and long, thin ramified processes that is typical under physiological conditions, to a less ramified morphology with fewer and thicker processes associated with pathological conditions, to an amoeboid, macrophage-like morphology that is characteristic of a fully activated microglial cell (Fig. 1). These morphological changes are accompanied by increased proliferation and phagocytic activity, changes in gene expression and motility, and release of numerous pro- and anti-inflammatory mediators (Corps et al., 2015; Mosser et al., 2017). Activation also upregulates microglial expression of the lysosomal protein CD68 and the 18 kDa mitochondrial translocator protein (TSPO), both of which are used as biomarkers of activated microglia (Table 1). Activated microglia perform numerous macrophage-like functions, including phagocytosis, antigen presentation, migration to sites of injury, and recruitment of additional immune cells via release of soluble mediators (Dantzer et al., 2008; Davalos et al., 2005; Nimmerjahn et al., 2005).

A hallmark characteristic of microglial activation is upregulated transcription and translation of cytokines and chemokines (Becher et al., 2017; Zhou et al., 2006; see also Table 1; Fig. 1). These soluble mediators function as an intercellular communication system to coordinate the neuroinflammatory response. Chemokines released by activated microglia, such as monocyte chemoattractant protein 1 (MCP-1) and macrophage inflammatory protein 1 (MIP-1α), establish a chemoattractant gradient to recruit other immune cells to the site of injury. Activated microglia also release both proinflammatory (IL-1β, IL-1α, IL-6, IFN-γ) and anti-inflammatory (IL-10, IL-4, TGF-β, arginase) cytokines, and the balance between these dictates the inflammatory tone (Frank-Cannon et al., 2009; Loane et al., 2014; Turtzo et al., 2014; Wang et al., 2013). Activation also increases microglial release of ROS and RNS. While physiologic levels of ROS and RNS are important signaling molecules in the brain (Kennedy et al., 2012; Massaad and Klann, 2011), excessive or sustained levels that

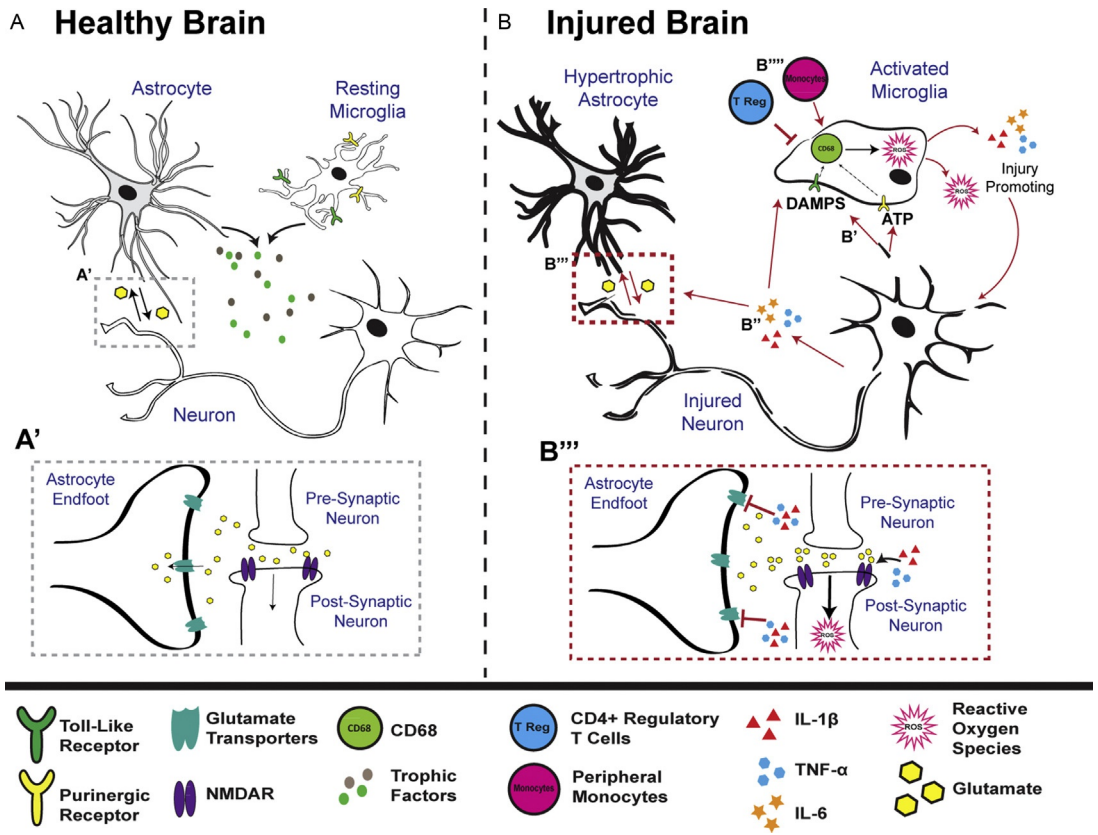

Fig. 1 See legend on opposite page.

overwhelm cellular antioxidant systems can lead to oxidative and nitrative damage to macromolecules, which in turn can cause cellular damage and elicit robust neuroinflammatory responses (Biswas, 2016; Loane et al., 2014).

Astrocytes, which are the predominant cell type in the CNS, are considered to be "pivotal regulators" of neuroinflammation because, depending on context and location, they can either promote neuroinflammation or protect healthy cells from cytotoxic byproducts of neuroinflammation. In response to brain injury, astrocytes undergo a reversible process known as reactive astrogliosis, which is associated with a hypertrophied cellular morphology that coincides with increased expression of the cytoskeletal proteins, glial fibrillary acidic protein (GFAP) and vimentin (Jensen et al., 2013; Kimelberg and Nedergaard, 2010; Liedtke et al., 1996). Activated astrocytes migrate toward and surround the site of injury to form a glial scar, which creates a barrier to prevent damage from spreading into surrounding healthy tissue (Jensen et al., 2013; Pekny et al., 2016). Activated astrocytes within this barrier provide trophic support to promote repair of the damaged tissue (Jensen et al., 2013). Reactive astrocytes can also amplify the neuroinflammatory response by signaling to neighboring cells via release of proinflammatory cytokines and chemokines (Fig. 1).

Oligodendrocytes are the major myelinating glia in the CNS. While damaged myelin has long been recognized as a trigger for microglial

Fig. 1 Schematic of neuroinflammation in the brain. (A) In the healthy brain, both astrocytes and resting microglia provide neurotrophic support to neurons to maintain homeostasis. (A′) Astrocytes modulate glutamatergic neurotransmission in the brain in part through uptake of synaptic glutamate. (B) Acute injury alters signaling between neurons, microglia and astrocytes. (B′) Injured neurons release ATP and damage associated molecular patterns (DAMPs) that bind to purinergic receptors or toll-like receptors (TLRs), respectively, to activate proximal microglia. Activated microglia become amoeboid in shape and increase expression of the lysosomal marker, CD68. Phagocytically active microglia produce elevated levels of ROS and inflammatory cytokines (IL-1β, TNF-α, IL-6) that promote further neurotoxic injury. (B″) Injured neurons also release inflammatory cytokines that trigger reactive astrogliosis as well as microglial activation. (B‴) IL-1β and TNF-α enhance glutamatergic signaling by increasing NMDAR activity and inhibiting glutamate transporters, which collectively promote excitotoxicity-induced ROS production. (B⁗) Breakdown of the blood brain barrier enables peripheral immune cells (T-cells and monocytes) to infiltrate the brain parenchyma and either promote or suppress further immune cell activation depending on the inflammatory context. This image is licensed under a Creative Commons Attribution 3.0 Unported License (Servier Medical Art by Servier and Creative Commons Attribution—Share Alike 4.0 International license).

activation (Clemente et al., 2013; Goldmann and Prinz, 2013; Williams et al., 1994), oligodendrocytes have largely been viewed as passive victims of neuroinflammation. Oligodendrocytes have a high metabolic rate and relatively few endogenous antioxidant defense systems, which makes them particularly vulnerable to increased ROS and RNS produced by sustained or excessive neuroinflammation (Thorburne and Juurlink, 1996). However, emerging evidence challenges this model, arguing instead that oligodendrocytes participate actively in the neuroinflammatory response. In response to injury or inflammatory stimuli, oligodendrocytes upregulate TLR subtypes and produce complement molecules, chemokines, and cytokines that function to coordinate the injury response by signaling to other cells, such as microglia (Balabanov et al., 2007; Bsibsi et al., 2002; Ramesh et al., 2012; Zajicek et al., 1995). Oligodendrocytes have also been demonstrated to produce soluble mediators that modulate microglia activity toward a less inflammatory tone (Peferoen et al., 2014).

Innate and adaptive immune cells of the peripheral immune system, including T cells, B cells, monocytes, and mast cells, play complicated roles in neuroinflammation. In situations of excessive neuroinflammation or compromised blood brain barrier (BBB) integrity, such as is observed in various neurologic disorders, peripheral immune cells that infiltrate the brain parenchyma can perpetuate local neuroinflammatory responses, thereby exacerbating tissue damage (Prinz and Priller, 2017). Conversely, migration of T cells into the brain parenchyma across an intact BBB in response to CNS injury can dampen neuroinflammation by producing neurotrophic factors and cytokines that modulate microglia and astrocyte activity to limit their release of neurotoxic factors, which restricts the spread of neuronal damage (Beers et al., 2008; Schwartz et al., 2003; Shaked et al., 2004).

Given the number of cell types and soluble mediators involved, it is perhaps not surprising that a wide variety of biomarkers are used to detect and quantify neuroinflammation (Table 1). In many cases, microglial and astrocytic activation are assessed by quantifying expression levels or the number of cells expressing biomarkers for microglia and/or reactive astrocytes. However, the pathophysiological relevance of increased expression of these biomarkers is not always clear. For example, upregulation of GFAP can be associated with neuroprotective or neurotoxic activity (Dallerac and Rouach, 2016). Expression of ionized calcium-binding adapter molecule 1 (Iba-1), which is used as a biomarker of microglial cells, does not distinguish between non-inflammatory vs. inflammatory microglia. Early studies distinguished "resting" from "activated" microglia based on cellular

morphology (ramified vs. amoeboid, respectively). However, accumulating evidence shows that microglia assume a variety of intermediate morphologies, which are not always linked to an inflammatory role, and the morphology of microglia in the developing brain differs from that observed in the mature brain under both physiologic and pathologic conditions (Mosser et al., 2017). Another common approach for quantifying neuroinflammation is to measure levels of soluble pro- or anti-inflammatory mediators in whole brain. However, this approach cannot distinguish between soluble mediators derived from activated microglia and/or astrocytes vs. those released from oligodendrocytes or peripheral immune cells. Further complicating interpretation, the effect of cytokines is context-dependent such that classic proinflammatory cytokines can have anti-inflammatory effects later in the inflammatory response. Despite these challenges, the currently available body of literature strongly suggests that neuroinflammation may be an important mechanism of OP neurotoxicity, based in large part on clinical and experimental evidence that multiple OPs upregulate one or more mediators of neuroinflammation.

3. Neuroinflammatory responses to neurotoxic OP exposures

Much of the initial data describing neuroinflammatory responses to OPs were generated from preclinical models of acute intoxication with OP nerve agents at doses that induced SE and other signs of cholinergic crisis (Table 2). Subsequently, evidence began to emerge demonstrating that acute intoxication with OP pesticides and even repeated low-level exposures to OP nerve agents or pesticides can also increase neuroinflammation. A general observation emerging from this expanding literature is that the neuroinflammatory response profile varies depending on the OP, the exposure scenario, and the time after exposure when inflammation is assessed (Banks and Lein, 2012; Damodaran and Abou-Donia, 2000; Grauer et al., 2008; Liu et al., 2012).

3.1 Acute OP intoxication increases neuroinflammation

In some of the earliest studies of OP-induced neuroinflammation, acute soman intoxication was observed to significantly increase GFAP at the mRNA and protein level, and to increase the number of amoeboid microglia in the piriform cortex, amygdala, and hippocampus within hours after the onset of SE (Baille et al., 2005; Collombet et al., 2005a; Zimmer et al., 1997).

Table 2 Acute OP intoxication triggers neuroinflammation.

Type of OP	Chemical	Model	Exposure paradigm	Neuroinflammatory response	Brain regions affected	Reference
Nerve Agent	Soman	Rat	77.7 μg/kg (im[a])	↑ GFAP within 1 h ↑ Number of OX-42+ cells and morphologic activation within 4 h	Piriform Cortex Hippocampus	Zimmer et al. (1997)
	Soman	Rat	99–126 mg/kg (sc[b]) (pre) HI-6[c], 125 mg/kg (ip[d]) 6–24 h	↑ *GFAP* mRNA (in situ hybridization)	Hippocampus	Baille-Le Crom et al. (1995)
	Soman	Rat	180 μg/kg (sc) (pre) HI-6, 125 mg/kg (ip) (post) AMN[e], 2–4 mg/kg (im)	↑ IB-4+ cells ↑ GFAP at 28 DPE[i]; ↑ *TNF-α* (2 h), *VCAM* (2 h), *IL-1β* (6 h), *E-Selectin* (6 h), *IL-6* (6 h), *ICAM* (6 h) mRNA—resolves within 48 h ↑ IL-1α (microglia), IL-1β (microglia), TNF-α, IL-6 (neurons) protein peaks at 12 h ↑ CXCL1 (neurons + endothelial cells), MIP-1α (neurons) protein peaks at 12 h and precedes neutrophil infiltration into brain	Piriform Cortex Hippocampus Thalamus	Finkelstein et al. (2012), Williams et al. (2003), Dillman et al. (2009), Johnson and Kan (2010), and Johnson et al. (2011)
	Soman	Rat	133 μg/kg (sc) (post) AMN, 2 mg/kg (im)	↑ GFAP at 48 h ↑ ILB_4 + cells at 48 h	Piriform Cortex Hippocampus Thalamus Amygdala	Angoa-Perez et al. (2010)
	Soman	Rat	100 μg/kg (sc)	↑ *IL-1β* mRNA at 30 min ↑ IL-1β protein peaks at 6 h and expressed in astrocytes and endothelial cells	Striatum Hippocampus Cerebellum Frontal Cortex	Svensson et al. (2001)
	Soman	Mouse	110 μg/kg (sc) (post) AMN, 5 mg/kg (ip)	↑ Microglial cell number peaking at 3 DPE ↑ But delayed GFAP immunoreactivity 3–90 DPE; no change in oligodendrocyte health at any time point	Hippocampus Amygdala	Collombet et al. (2005a)

	Soman	Mouse	172 μg/kg (sc) (pre) HI-6, 50 mg/kg (ip)	↑ *IL-1β, TNF-α, IL-6, SOCS3, ICAM* mRNA as early as 1 h in cortex but delayed up to 6 h in hippocampus. Does not resolve for 7 DPE	Cortex Hippocampus Cerebellum	Dhote et al. (2007)
	Sarin	Rat	50 μg/kg/mL (im)	↑ *GFAP* and *vimentin* (northern blot) as early as 1 h in cortex, cerebellum and midbrain but not until 3 days in the brainstem; persisted until 7 days in all four brain regions ↑ *Prostaglandin* (microarray) at 2 h	Cortex Cerebellum Brainstem Midbrain	Damodaran et al. (2002, 2006)
	Sarin	Rat	108 μg/kg (sc) (post) AS[f], 2 mg/kg (im) 2-PAM[g], 25 mg/kg (im) Diazepam,	↑ *TNF-α, IL-1β, IL-10, IL-6, Neurotrophin, TREM1* mRNA within 1 h (peaks by 6 h); does not resolve by 24 h.	Piriform Cortex Hippocampus Thalamus Amygdala	Spradling et al. (2011a,b)
	Sarin	Rat	108 μg/kg (im) (pre) PB[h], 0.1 mg/kg (im); HI-6, 5 mg/kg (im) (post) AS—2 mg/kg (im) Midazolam—1 mg/kg (im)	↑ IL-1β, IL-6, PGE2 protein within 2 h, peaking between 2 and 24 h. Protein levels correlate with seizure duration	Piriform Cortex Hippocampus	Chapman et al. (2006)
Pesticide	Paraoxon	Rat	0.45 mg/kg (im) (post) AS—3 mg/kg (im); Toxogonin—20 mg/kg (im)	↑ IB-4+ cell number at 28 DPE ↑ GFAP at 28 DPE	Piriform Cortex	Finkelstein et al. (2012)
	DFP	Rat	9 mg/kg (ip) (pre) PB, 0.1 mg/kg (im); AMN, 20 mg/kg (im)	↑ *GFAP* mRNA at 24 h post DFP, ↑↑ by 3 DPE ↑ GFAP with distinct spatiotemporal progression beginning as early as 1 h with biphasic response that persists for 21 DPE ↑ CD11b+ cell number and morphologic activation at 24 h ↑ IBA-1 and morphological activation persists for 14 DPE, ↑at 21 DPE ↑ TSPO peaks at 7 DPE in whole brain	Piriform Cortex Hippocampus Amygdala Thalamus	Liu et al. (2012), Li et al. (2015), and Flannery et al. (2016)

Continued

Table 2 Acute OP intoxication triggers neuroinflammation.—cont'd

Type of OP	Chemical	Model	Exposure paradigm	Neuroinflammatory response	Brain regions affected	Reference
	DFP	Rat	9 mg/kg (ip) (pre) PB, 0.1 mg/kg (im); AMN, 20 mg/kg (im)	↑ *IL-1β, IL-6* mRNA at 24 h No change *TNF-α* mRNA at 24 h	Hippocampus	Li et al. (2015)
	DFP	Rat	3.2 mg/kg (sc) (pre) PB, 0.026 mg/kg (im) (post) AMN, 2 mg/kg (ip); 2-PAM, 25 mg/kg (im)	↑ GFAP at 3 DPE ↑ IBA-1at 3 DPE	Hippocampus Amygdala	Kuruba et al. (2018)
	DFP	Rat	4 mg/kg (sc) (pre) PB, 0.1 mg/kg (im) (post) AS, 2 mg/kg (im); 2-PAM, 25 mg/kg (im)	↑ GFAP and IBA-1 immunoreactivity with varying spatiotemporal progression-persists for 60 DPE	Piriform Cortex Hippocampus Cerebral Cortex Amygdala Thalamus	Siso et al. (2017)
	DFP	Rat	4.5 mg/kg (sc) (pre) PB, 0.1 mg/kg (im) (post) AMN, 2 mg/kg (im); 2-PAM, 25 mg/kg (im)	↑ TNF-α, IL-1β, IL-6, KC/GRO proteinat 24 h	Hippocampus Piriform Cortex	Liang et al. (2018)

[a]im—intramuscular.
[b]sc—subcutaneous.
[c]HI-6—asoxime chloride.
[d]ip—intraperitoneal.
[e]AMN—atropine methyl nitrate.
[f]AS—atropine sulfate.
[g]2-PAM—2-pyridine aldoxime methylchloride.
[h]PB—pyridostigmine bromide.
[i]DPE—day post exposure.

These early observations were subsequently replicated in other preclinical models of acute intoxication with soman (Angoa-Perez et al., 2010) or sarin (Damodaran et al., 2002, 2006). For both OP nerve agents, the temporal patterns of rapid astrogliosis and microglial activation following exposure varied regionally but generally peaked within 24–72 h and then subsided. Observations along an extended timeline post-exposure in a mouse model of acute soman intoxication revealed a second wave of increased GFAP expression in the medial and lateral septum and hippocampus of mice beginning 3 days after exposure that persisted for up to 30–90 days depending on the brain region (Collombet et al., 2005a).

Consistent with observations of astrogliosis and microglial activation, transcriptomic analysis of brains from rats intoxicated with sarin identified signaling pathways associated with inflammation among the most significantly altered gene expression changes (Spradling et al., 2011a). Regional analyses of cytokine expression in the brain at the mRNA or protein level indicated that the spatiotemporal patterns varied between cytokines (Table 2). Transcript levels of TNF-α peaked in most brain regions within 2 h following soman intoxication, whereas increased mRNA expression of other cytokines, such as IL-1β and IL-6, was delayed, with significant upregulation not observed until 6 h post-exposure and even then only in very specific brain regions, such as the piriform cortex (Dhote et al., 2007; Dillman et al., 2009; Svensson et al., 2001; Williams et al., 2003). Analyses of the protein expression of cytokines (TNF-α, IL-1β, IL-6), prostaglandins and chemokines (MCP-1, MIP-1α) similarly indicated a robust but transient response in the brain following soman and sarin exposures (Angoa-Perez et al., 2010; Chapman et al., 2006; Johnson and Kan, 2010; Johnson et al., 2011). More detailed spatial resolution of the expression of these inflammatory mediators by immunohistochemical analyses revealed that neurons, microglia, astrocytes, and components of the BBB vasculature all expressed these mediators to varying degrees (Johnson and Kan, 2010; Johnson et al., 2011).

More recent preclinical research on OP-induced neuroinflammation has focused on acute poisoning with seizurogenic doses of the OP pesticides diisopropylfluorophosphate (DFP) or paraoxon (Table 2). Multiple laboratories have demonstrated that acute intoxication with paraoxon (Finkelstein et al., 2012) or DFP (Ferchmin et al., 2014; Flannery et al., 2016; Kuruba et al., 2018; Li et al., 2015; Liu et al., 2012; Rojas et al., 2015) activates astrocytes and microglia as indicated by significantly increased expression of GFAP and morphological assessment of the activation state of Iba-1 immunopositive cells, respectively. Acute DFP intoxication also elevates

mRNA and protein expression of the proinflammatory cytokines, IL-1β, TNF-α, and IL-6 (Li et al., 2015; Liang et al., 2018) and prostaglandins (Rojas et al., 2015) within 1 day after DFP exposure. A contemporary translational tool for assessing neuroinflammation—positron emission tomography (PET) imaging of the 18 kDa mitochondrial translocator protein (TSPO)—has also been employed to longitudinally monitor the neuroinflammatory response in the rat model of acute DFP intoxication. TSPO is typically expressed at low levels on the outer mitochondrial membrane of glial cells in the brain; however, upon activation, TSPO expression is significantly upregulated, making it a useful biomarker of microglial activation, and perhaps reactive astrocytes (Guilarte, 2018; Lavisse et al., 2012; Veiga et al., 2007). In general concurrence with immunohistochemical evidence of neuroinflammation, PET imaging of TSPO labeling in the brains of DFP intoxicated rats indicated significantly increased neuroinflammation in the hippocampus and cortex relative to VEH controls at 2, 7 and 21 days, with peak binding of the TSPO ligand, [^{11}C]PK11195 observed at 7 days post-exposure (Flannery et al., 2016).

As observed with OP nerve agents, glial activation was seen within hours to days following DFP or paraoxon exposure (Finkelstein et al., 2012; Flannery et al., 2016; Liu et al., 2012; Siso et al., 2017). But relative to observations with OP nerve agents, this cellular response peaked much later (3–7 days post-exposure). Also in contrast to observations with OP nerve agents, reactive astrogliosis in the DFP model preceded microglial activation, at least in the two brain regions where this relationship was examined, the hippocampus and cortex (Flannery et al., 2016). The neuroinflammatory responses to acute DFP intoxication have been shown to persist for at least 30–60 days post-exposure; however, the temporal profile varied across brain regions (Flannery et al., 2016; Siso et al., 2017). Reactive astrogliosis persisted significantly longer in the cortex relative to the hippocampus, and while a second wave of microglial activation was apparent in both brain regions at 7 days post-exposure, this persisted significantly longer in the hippocampus relative to the cortex. Whether the second wave of apparent microglial cell activation represented microglia and/or monocytes recruited from the periphery was not addressed in this study.

3.2 Evidence that low-level OP exposures also trigger neuroinflammatory responses

There is growing consensus that repeated low-level OP exposures are linked to neurobehavioral deficits in humans following either occupational

exposures (Meyer-Baron et al., 2015; Rohlman et al., 2011) or developmental exposures (Bouchard et al., 2011; Burke et al., 2017; Engel et al., 2011). But the question of whether these OP effects are associated with neuroinflammation has only recently been addressed. The data emerging from preclinical models suggest that repeated low-level OP exposures trigger neuroinflammatory responses (Table 3).

The majority of these studies have focused on the OP pesticide chlorpyrifos. In adult mice, repeated administration of chlorpyrifos at levels that did not inhibit red blood cell cholinesterase activity upregulated GFAP protein expression in the hippocampus (Lim et al., 2011). Genes in the IL-6 and TNF-α signaling pathways were upregulated in the forebrain of adult rats exposed to chlorpyrifos at levels that inhibited AChE, but did not cause cholinergic symptoms (Stapleton and Chan, 2009). In a model of developmental chlorpyrifos exposure, GFAP protein was increased in the brain of fetal animals following chlorpyrifos exposure of the pregnant dam on gestational day 17 through 20 at doses that induced systemic toxicity, but not cholinergic symptoms (Garcia et al., 2002). In contrast, postnatal administration of chlorpyrifos to the dam caused an initial sex-specific decrease in GFAP expression in pups followed by a rebound of GFAP expression to levels significantly higher than vehicle controls at 15 days after the last exposure (Garcia et al., 2002). The authors postulated that elevated gestational GFAP reflected chlorpyrifos-induced astrogliosis, whereas postnatal GFAP deficits were caused by depression of cell differentiation during periods of glial proliferation. A similar decrease in GFAP expression was observed in developing rat pups following a single exposure to chlorpyrifos (2 mg/kg, sc) on postnatal day 7 (Ray et al., 2010). Collectively, these observations suggest that astrocytes in the developing brain are susceptible to the toxic effects of chlorpyrifos, but that the outcome, at least in terms of GFAP expression, varies quantitatively and qualitatively as a function of sex and the developmental age at the time of exposure. In other studies of developmental chlorpyrifos exposure, chlorpyrifos in the maternal diet throughout gestation and lactation upregulated the proinflammatory cytokine IL-1β, but downregulated the anti-inflammatory cytokine IL-10 in the hippocampus of weanling rat pups (Gomez-Gimenez et al., 2017). Increased IL-1β levels in the hippocampus correlated negatively with pup performance in a spatial learning task. Direct exposure of pups to chlorpyrifos during the postnatal period was also sufficient to promote neuroinflammation in the amygdala, as evidenced by increased expression of Iba-1 and proinflammatory cytokines (Tian et al., 2015). The potential relevance of these findings to

Table 3 Repeated Low-level OP exposure upregulates biomarkers of neuroinflammation.

Type of OP	Model	Exposure Paradigm	Neuroinflammatory Response	Brain Regions Affected	Reference
Sarin	Adult rat	0.2 or 0.4 mg/m^3, inhalation 1 h/day for 1 or 5 days	Dose-dependent ↑ *IL-1β*, *TNFα*, *IL-6* mRNA determined by RT-PCR of whole brain after 5 days of daily exposure	whole brain	Henderson et al., 2002
Chlorpyrifos	Adult rat	0.5, 1.0, 5, 10, 30, 50 mg/kg single exposure by oral gavage	Microarray analyses indicated disruption of IL-6 and TNFα signaling pathways at 96 h post-exposure	Forebrain	Stapleton and Chan, 2009
Chlorpyrifos	Adult mouse	20 or 40 mg/kg, dermal application to the tail for 7 d	↑ GFAP immunoreactivity determined by IHC[a]	Hippocampus	Lim et al., 2011
Chlorpyrifos	Developing rats (prenatal)	1, 2, 5, 10, 20 or 40 mg/kg/d Dams injected sc daily Gestational days 17-20	↑ GFAP in 20 and 40 mg/kg dose groups as determined by WB[b] at gestational day 21	Forebrain Midbrain and brainstem	Garcia et al., 2002
Chlorpyrifos	Developing rats (postnatal)	1 or 5 mg/kg/d Dams injected sc daily PND[c] 1-4 or PND 11-14	↓ GFAP in cerebellum of male but not female pups at PND 5 following exposure PND 1-4 ↑ GFAP in multiple brain regions of male, but only striatum in female at PND 30 following exposure PND 11-14	Cerebellum (PND 5) Multiple brain regions (PND 30)	Garcia et al., 2002
Chlorpyrifos	Neonatal rat	5 mg/kg, intrahepatic injection Daily, PND 11-14	↑ *TNFα*, *IL-6* mRNA determined by qRT-PCR ↑ HMGB1[d] and Iba-1 immunoreactivity determined by IHC and WB at 12 (Iba-1 only), 24 and 72 h after last injection	Amygdala	Tian et al., 2015

Chlorpyrifos	Developing rats	0.1, 0.3 or 1 mg/kg Daily in maternal diet Gestational day 7 – PND 21	All doses ↑ IL-β in male but not female pups; 0.3 and 1.0 mg/kg ↓ IL-10 in female but not female pups determined by WB	Hippocampus	Gómez-Giménez et al., 2017
Dichlorvos	Adult rat	2.5 mg/kg, sc[e] Daily for 12 weeks	↑ MAC expression as determined by IHC, WB of brain homogenates and immunostaining of isolated microglial cells; ↑ IL-1β, IL-6 , TNFα as determined by IHC and ELISA	Substantia nigra Corpus striatum	Binukumar et al., 2011
Dimethoate	Adult mouse	1.4 mg/kg, ip[f] 3 times a week for 5 weeks	↑ *TNFα*, *IL-6* mRNA determined by qRT-PCR ↑ Microglial cell activation determined by morphological assessment of Iba-1 immunopositive cells	Hippocampus (cytokines) DG[g], substantia nigra (microglia)	Astiz et al., 2013
Malathion	Adult mouse	30 or 100 mg/kg, sc Daily for 15 d	Both doses ↑ GFAP immunoreactivity determined by IHC	Hippocampus (CA1, CA3, DG)	Dos Santos et al., 2016
Malathion	Adult mouse	200 mg/kg, oral gavage Daily for 8 weeks	↑ Number of Iba-1 immunoreactive cells	Substantia nigra	Ahmed et al., 2017

[a]IHC – immunohistochemistry;
[b]WB – western blotting;\
[c]PND – postnatal day;
[d]HMGB1 – high mobility group box 1;
[e]sc – subcutaneous;
[f]ip – intraperitoneal;
[g]DG – dentate gyrus

the developing human brain is demonstrated in a study showing that *in vitro* exposure of human fetal astrocytes to chlorpyrifos for 1 week increased levels of *GFAP, IL-6*, and other genes associated with proinflammatory cytokine signaling pathways (Mense et al., 2006).

Studies of repeated low-level exposures in adult rodents to OPs other than chlorpyrifos confirm that neuroinflammation is not a response unique to chlorpyrifos (Table 3). For example, in rats exposed to repeated low doses of sarin vapor, brain levels of transcripts for *TNF-α*, *IL-1β* and *IL-6* were significantly increased (Henderson et al., 2002). In another study, rats were administered the OP pesticide dichlorvos for 12 weeks at 2.5 mg/kg/day, a dosing paradigm previously shown to inhibit AChE by 10–55%, but not cause marked signs of cholinergic toxicity (Verma et al., 2009). At the end of the exposure, significant microglial activation was observed in the substantia nigra and corpus striatum (Binukumar et al., 2011). In this model, increased microglial activation preceded neuronal degeneration in the same brain regions and was accompanied by increased levels of proinflammatory cytokines. Two additional OP pesticides, dimethoate and malathion, have been demonstrated to increase biomarkers of neuroinflammation in adult mice exposed to repeated doses that did not cause cholinergic toxicity. Dimethoate increased expression of *TNF-α* and *IL-6* mRNA in the hippocampus and increased the number of activated microglia in the dentate gyrus and substantia nigra, as determined by morphological analyses of Iba-1 immunopositive cells (Astiz et al., 2013). At doses (30 and 100 mg/kg) that inhibited hippocampal AChE by 40% but did not cause cholinergic toxicity, malathion increased GFAP immunoreactivity in the hippocampus coincident with impaired spatial memory and discrimination (Dos Santos et al., 2016). At a higher dose (200 mg/kg), malathion caused significant dopaminergic neurodegeneration in the substantia nigra and deficits in locomotor behavior and significantly increased the number of activated microglia in the substantia nigra (Ahmed et al., 2017).

4. How do OPs trigger neuroinflammatory responses?

4.1 Cholinergic mechanisms of OP-induced neuroinflammation

A key question is whether neuroinflammatory responses to OP intoxication are mechanistically related to AChE inhibition. AChE inhibition is the principal mechanism of OP-induced cholinergic crisis, which is associated with a robust inflammatory response (Table 2). However, results from diverse models of OP intoxication provide a compelling argument against

cholinergic mechanisms of OP-induced neuroinflammation. Particularly convincing are data obtained from a preclinical model of Gulf War Illness (O'callaghan et al., 2015) in which adult male mice are administered corticosterone in the drinking water for 4 days at 400 mg/mL, a level associated with high physiological stress to replicate Gulf War theater conditions, prior to injection with DFP (4 mg/kg, ip). This exposure paradigm resulted in brain-wide neuroinflammation as assessed by qPCR of proinflammatory cytokines, and Western blot analyses of phosphorylated and total STAT, a signaling molecule activated by inflammatory mediators. To test the role of AChE inhibition in the neuroinflammatory response, animals were administered corticosterone plus either irreversible (chlorpyrifos oxon or DFP) or reversible (pyridostigmine bromide or physostigmine) inhibitors of AChE (Locker et al., 2017). Chlorpyrifos oxon, and DFP alone caused cortical and hippocampal neuroinflammation and activation of STAT, which were markedly enhanced by pretreatment with corticosterone. In contrast, exposure to the reversible AChE inhibitors alone or with corticosterone pretreatment did not cause neuroinflammation or activate STAT. All of the CNS-acting AChE inhibitors (DFP, chlorpyrifos oxon, and physostigmine) decreased brain AChE activity to an equivalent level in the absence of corticosterone; however, pretreatment with corticosterone abrogated this effect only for the irreversible inhibitors. The lack of correlation between the level of AChE inhibition and the magnitude of neuroinflammation in this model suggests that neuroinflammation was mediated by non-cholinergic mechanisms (Locker et al., 2017). This conclusion was further supported by measurement of acetylcholine levels in specific brain regions, which suggested that the exacerbated neuroinflammatory effects observed in the brains of animals treated with corticosterone and DFP were not driven by accumulation of acetylcholine in the brain (Miller et al., 2018). The second line of evidence are studies demonstrating that repeated low-level OP exposures induce inflammation even in the absence of seizures or significant AChE inhibition (Table 3). For example, repeated low-level doses of sarin elevated cytokine levels in rat brain in the absence of central AChE inhibition, although blood ChE activity was reduced (Henderson et al., 2002). Similarly, chronic chlorpyrifos administration increased GFAP expression in the hippocampus of rats without inhibiting serum cholinesterase (Lim et al., 2011). Observations such as these indicate that prolonged low-level exposure to OPs can trigger neuroinflammatory responses in the absence of AChE inhibition and overt clinical symptoms of OP poisoning.

The mechanism(s) by which the Gulf War Illness exposure paradigm and the repeated low-level OP exposures induce neuroinflammation have yet to

be defined, but one possibility is that OPs interact directly with inflammatory cells to trigger the release of inflammatory mediators. There is experimental evidence to support this hypothesis. For example, the OP pesticide parathion stimulated release of TNF-α from primary alveolar macrophages isolated from guinea pig lung and exposed *ex vivo* (Proskocil et al., 2013). Exposure of human whole blood cultures to the OP chlorpyrifos potentiated LPS-induced release of IFN-γ (Duramad et al., 2006). Gene and protein expression profiling of primary cultures of human fetal astrocytes revealed that chlorpyrifos upregulated GFAP and key inflammatory mediators, including IFN-γ and IL-6 (Mense et al., 2006). The molecular mechanisms by which OPs activate immune cells remain largely unknown; however, recent studies demonstrated that stimulation of cholinergic receptors as a consequence of acetylcholine accumulation at immunological synapses was sufficient to activate signaling cascades that evoked inflammatory responses in macrophages (Mitra et al., 2017). While these studies demonstrate that OPs can directly activate peripheral immune cells, it is unknown whether this phenomenon holds true in the brain.

4.2 Is OP-induced neuroinflammation driven by oxidative stress?

Increased brain expression of biomarkers of oxidative stress has been reported in preclinical models of acute OP poisoning (Kim et al., 1999; Liang et al., 2018; Zaja-Milatovic et al., 2009) and repeated low-level OP exposure (reviewed in Soltaninejad and Abdollahi, 2009). Such observations have led many to propose that OP-induced neuroinflammation is directly related to increased oxidative stress (Milatovic et al., 2006; Naughton and Terry, 2018; Pearson and Patel, 2016; Soltaninejad and Abdollahi, 2009). This hypothesis derives in large part from evidence indicating that the excessive cholinergic neurotransmission caused by AChE inhibition following acute OP intoxication increases glutamatergic signaling, which is critical for sustaining seizures initiated by cholinergic mechanisms (Mcdonough and Shih, 1997). This excessive glutamatergic signaling is thought to drive early neuroinflammatory responses via at least two mechanisms (Fig. 2). First, excessive release of neuronal glutamate can cause astroglial dysfunction, which stimulates reactive astrogliosis (reviewed in Viviani et al., 2014). Second, sustained activation of NMDA receptors can cause excitotoxicity as a result of excessive intracellular Ca^{2+} and mitochondrial stress that elevate ROS to levels that are neurotoxic (Arundine and Tymianski, 2003). Neuronal cell death as a result of either necrosis or apoptosis releases signals, such as ATP, that activate

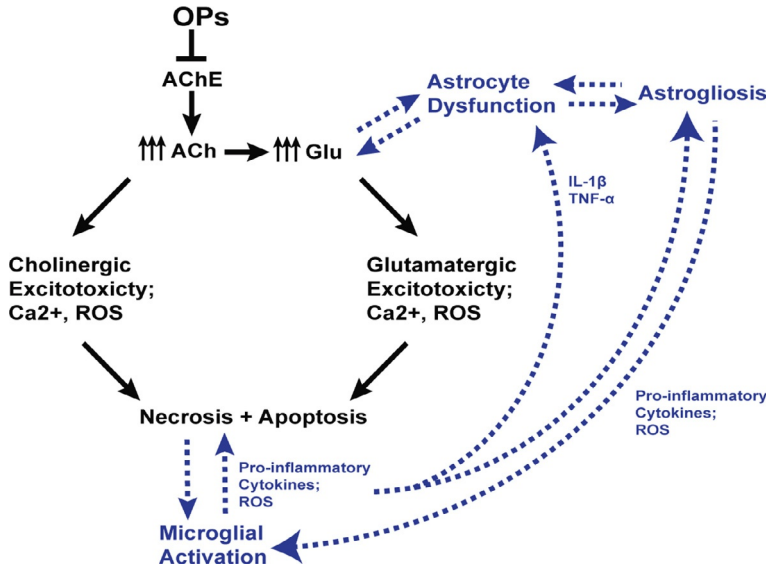

Fig. 2 Mechanisms by which acute OP intoxication triggers neuroinflammation. OPs inhibit acetylcholinesterase (AChE) leading to excessive build-up of acetylcholine (ACh) in cholinergic synapses. During the immediate response (~5 min), cholinergic excitotoxicity is characterized by excessive Ca^{2+} influx and increased ROS production, which ultimately trigger necrotic and apoptotic cell death. Hypercholinergic signaling also activates glutamatergic signaling in the brain, leading to glutamate-induced excitotoxicity. Necrotic cell death releases damage associated molecular patterns (DAMPs) that trigger release of inflammatory cytokines and ROS from activated microglia. These pro-inflammatory mediators can perpetuate the neurotoxic response as well as activate surrounding astrocytes to undergo reactive astrogliosis. Excessive or prolonged neuroinflammation can drive astrocyte dysfunction that promotes glutamate-mediated excitotoxicity. This image is licensed under a Creative Commons Attribution 3.0 Unported License (Servier Medical Art by Servier and Creative Commons Attribution—Share Alike 4.0 International license).

microglia (Chung et al., 2006; Joseph et al., 2005; Potts et al., 2006; Rahman et al., 2006; Wang et al., 2006).

It has long been proposed that the neuroinflammation observed in preclinical models of acute OP intoxication is a response to excitotoxic neurodegeneration. In support of this hypothesis, early neuroinflammatory responses were reported to coincide with neurodegeneration in preclinical models of acute OP intoxication (reviewed in Chen, 2012; Collombet, 2011; De Araujo Furtado et al., 2012; Pereira et al., 2014). Consistent with long-standing observations that the extent of neurodegeneration is directly correlated to the duration of OP-induced seizure activity

(Filliat et al., 1999; Hobson et al., 2017; Lemercier et al., 1983; Mcdonough and Shih, 1997; Siso et al., 2017), a strong positive correlation has recently been reported between seizure activity and various indices of neuroinflammation. Specifically TSPO labeling in the brain as detected by PET imaging of rats acutely intoxicated with the OP pesticide DFP (Flannery et al., 2016) and expression of neuronal cyclooxygenase 2 (COX-2) in the hippocampus, amygdala, piriform cortex, and thalamus of rats acutely intoxicated with the OP nerve agent soman (Angoa-Perez et al., 2010) are positively correlated with seizure duration. However, data from the few studies that have examined spatiotemporal patterns of neurodegeneration and neuroinflammation in the same animal following acute OP intoxication suggest that neuroinflammation precedes neuronal cell death in multiple brain regions. Thus, increased GFAP immunoreactivity was detected in the piriform cortex, amygdala and dentate gyrus within 1 h after acute intoxication with DFP, which preceded any signs of neurodegeneration (Li et al., 2011; Liu et al., 2012). Similarly, in a preclinical model of acute soman intoxication, GFAP immunoreactivity was observed prior to evidence of neuronal injury (Baille-Le Crom et al., 1995; Zimmer et al., 1997). However, these observations do not rule out the possibility that neurodegeneration may trigger other aspects of neuroinflammation, such as microglial activation.

There are significantly fewer data regarding the temporal and mechanistic relationships of either neuroinflammation or neurodegeneration to oxidative stress induced by acute OP intoxication. A study of the spatiotemporal profile of the expression of oxidative stress biomarkers (specifically, the ratio of reduced to oxidized glutathione and levels of nitrosylated tyrosine) in rats acutely intoxicated with DFP reported increased oxidative stress in the brain at 24 and 48 h post-exposure, but not 6 and 12 h post-exposure (Liang et al., 2018). Considered in light of studies that have reported neuroinflammation in preclinical models of acute DFP intoxication at significantly earlier time points (Flannery et al., 2016; Rojas et al., 2015; Siso et al., 2017), these data suggest that neuroinflammation may be driving oxidative stress. However, treatment of rats acutely intoxicated with DFP with a catalytic antioxidant administered at 5 or 15 min post-exposure significantly attenuated both oxidative stress and neuroinflammation, the latter determined by levels of proinflammatory cytokines in the brain, and these effects coincided with decreased neurodegeneration (Liang et al., 2018). Antioxidant treatment has also been reported to attenuate neuroinflammation in preclinical models of repeated low-level OP exposure.

The 6-gingerol-rich fraction from *Zingiber officinale* (ginger) decreased TNF-α levels and neuronal apoptosis in the brain of rats chronically exposed to chlorpyrifos at a level (5 mg/kg daily for 35 days) that caused significant oxidative stress (Abolaji et al., 2017). Crocin, a water-soluble carotenoid isolated from the stigma of *Crocus sativus* (saffron), decreased TNF-α and IL-6 levels in the striatum of rats exposed to malathion (100 mg/kg, ip, daily for 28 days). This exposure paradigm caused neuropathological and behavioral phenotypes relevant to Parkinson's disease, and crocin treatment not only attenuated neuroinflammation but also blocked oxidative stress and improved performance on tests of locomotor behavior (Mohammadzadeh et al., 2018).

In summary, the existing data demonstrate that both acute OP intoxication and repeated low-level OP exposure cause oxidative stress in the brain. However, the data supporting oxidative stress as a principal mechanism driving OP-induced neuroinflammation are equivocal and the functional relationships between oxidative stress, neuroinflammation, and neurodegeneration are not clear. While experiments designed to specifically address this question are required, it seems likely that these are dynamic relationships that change depending on the OP exposure paradigm, the time post-exposure and the brain region(s) involved.

5. What is the pathophysiologic relevance of OP-induced neuroinflammation?

Preclinical studies have provided compelling evidence that both acute high-dose and repeated low-level exposures to OPs induce neuroinflammation. However, the relevance of neuroinflammation to the pathophysiology of neurological effects associated with OP exposure has yet to be determined. Critical questions include: Is neuroinflammation a cause or consequence of the neurotoxic effects of OPs? Is the neuroinflammatory response to OPs neurotoxic or neuroprotective in its actions?

The answers to these questions are emerging primarily from two areas of research: clinical and preclinical studies of neuroinflammation in the context of neurological diseases phenotypically similar to outcomes associated with neurotoxic OP exposures (Table 4), and mechanistic studies in preclinical models of OP neurotoxicity.

Table 4 Neuroinflammatory components implicated in pathogenesis of neurologic disorders.

Disease state	Neuroinflammatory component	Neuroinflammatory response (model)	References
Neurodegenerative disease	IL-1β; TNF-α Complement; Activated microglia	⬆ Pro-inflammatory cytokines, complement, microglia colocalized with beta-amyloid plaques in hippocampus (AD[a] human); ⬇ Inflammasome activation reduces IL-1β production and protects AD associated deficits in spatial memory (mouse); ⬇ microglial activation protects against deficits in spatial memory (TBI[b]—rat); ⬆ *TNF-α, IL-1β* polymorphisms associated with greater risk of developing PD[c] (human); ⬆ LPS-induced microglia activation and inflammation show selective dopaminergic neurotoxicity (PD—rat)	Reviewed in Simen et al. (2011), Heneka et al. (2015), Liu and Bing (2011), Wahner et al. (2007a,b), Walker and Tesco (2013)
Age-related, non-pathogenic cognitive decline	IL-6 IL-1; Innate immune response genes	⬆ IL-6 in serum inversely correlated with attention and working memory (human); ⬆ IL-1β in serum associated with decreased executive function (human); ⬆ IL-1 and microglia activation associated with impaired spatial learning (mouse; rat); ⬆ Inflammation-related gene expression inversely correlated with genes associated with learning and memory (rat)	Reviewed in Simen et al. (2011)

Depression/anxiety	TNF-α; IL-1β; IL-6; Activated microglia; Glial cell activation	⬆ TNF-α and IL-1β in CSF predictive of poor performance on psychometric tests for anxiety and depression (human); ⬆ microglia activation required for stress-induced anxiety (mouse; rat); ⬆ TSPO, IL-1β and IL-6 expression in brains of anxiogenic animals (rat); ⬆ *IL-1β* mRNA and protein associated with depressive-like behavior. Reduction in cytokine levels results in reversal of behavior (rat)	Rossi et al. (2017), Wang et al. (2018), Wohleb et al. (2014), Yue et al. (2018)
Epilepsy	IL-1β; COX2; Activated microglia; Astrogliosis	⬆ Alteration in phenotype, function and activation of microglia and astrocytes in hippocampus (human); ⬆ LPS-induced microglial activation results in downstream astrocytic enhancement of postsynaptic currents (mouse, in vitro); Inhibition of IL-1β signaling slows development of acquired epilepsy (mouse); Pharmacological inhibition of COX2 reduces microglial activation and the development of acquired epilepsy (mouse); ⬆ reactive astrogliosis in the absence of BBB[d] pathology triggers the development of acquired epilepsy (mouse); ⬇ TSPO expression and GFAP reactivity correlate with reduced incidence of acquired epilepsy (rat)	Review in Vezzani et al. (2013), Jung et al. (2006), Kleen et al. (2012), Pascual et al. (2012), Robel et al. (2015), Vezzani et al. (2000)

[a]AD – Alzheimer's' Disease;
[b]TBI – Traumatic Brain Injury;
[c]PD- Parkinson's Disease;
[d]BBB- Blood Brain Barrier

5.1 Neuroinflammation in neuropsychiatric disorders

Observations from clinical and preclinical studies of neurological diseases with phenotypic outcomes similar to those associated with neurotoxic OP exposures provide indirect support for the hypothesis that neuroinflammation is mechanistically linked to the neurological effects of OPs (Table 4). Acute OP intoxication that causes SE or other signs of cholinergic crisis is associated with delayed and persistent cognitive dysfunction, depression, anxiety, and spontaneous recurrent seizures in both humans and preclinical models (reviewed in Chen, 2012; De Araujo Furtado et al., 2012; Pereira et al., 2014). Similarly, repeated low-level OP exposures have been linked to cognitive deficits and neuropsychiatric conditions such as depression, anxiety, and suicidal tendencies (reviewed in Voorhees et al., 2016). Beyond the field of OP neurotoxicology, there is an extensive literature linking dysregulated neuroinflammation to neurological disease (Skaper et al., 2018), including cognitive deficits in neurodegenerative disease (Guedes et al., 2018; Hong et al., 2016; Mcafoose and Baune, 2009; Wyss-Coray and Rogers, 2012), cognitive impairment in socially defeated mice (Pfau and Russo, 2016), which may be particularly relevant to Gulf War Illness, depression and anxiety (Jeon and Kim, 2018; Kim and Jeon, 2018; Pfau et al., 2018), and electrographic abnormalities, including acquired epilepsy (Devinsky et al., 2013; Rana and Musto, 2018).

Persistent memory impairment is one of the most common neurological consequences observed in humans survivors of acute OP poisoning (Chen, 2012). Years after the 1995 Tokyo subway attack with sarin, exposed rescue personnel and subway workers still exhibited significant deficits on memory tests compared to non-exposed control subjects (Miyaki et al., 2005; Nishiwaki et al., 2001; Yanagisawa et al., 2006). Multiple lines of evidence implicate dysregulated neuroinflammation, especially in the hippocampus, in the pathogenesis of age-related cognitive dysfunction (Simen et al., 2011) and cognitive impairment associated with neurodegenerative disease (Heneka et al., 2015; Liu and Bing, 2011; Wahner et al., 2007a,b). Microglial activation seems particularly important in the pathogenesis of Alzheimer's disease (AD) (Heneka et al., 2015). A network-based integrative analysis of whole-genome expression profiling and genotypic data obtained from brains of individuals with late-onset AD and non-demented control subjects implicated the immune and microglial modules as the molecular systems most strongly associated with the pathophysiology of late-onset AD (Zhang et al., 2013). Consistent with this clinical finding, pharmacologic inhibition of microglial proliferation in a mouse model of

AD shifted microglia to an anti-inflammatory phenotype, prevented synaptic degeneration, and improved memory (Olmos-Alonso et al., 2016). Cognitive impairment following traumatic brain injury (TBI) has similarly been associated with neuroinflammation that persists for years after initial injury. Therapeutic strategies to prevent or mitigate microglial activation have shown efficacy in suppressing production of inflammatory mediators and improving long-term cognitive function in preclinical TBI models (Walker and Tesco, 2013).

Neuropsychiatric disorders, and in particular depression and anxiety, are common morbidities of acute OP intoxication in both humans and preclinical models (Coubard et al., 2008; Delgado et al., 2004; Levin et al., 1976; Okumura et al., 2005; Prager et al., 2014b; Savage et al., 1988). Clinical and experimental evidence implicates neuroinflammation as a significant contributing factor in the etiology of both these neuropsychiatric disorders (Dantzer et al., 2011; Jeon and Kim, 2018; Kim and Jeon, 2018; Pfau et al., 2018). Patients with major depression are reported to have significantly elevated blood levels of proinflammatory cytokines; conversely, non-depressed patients who received chemokine immunotherapy tended to develop clinical signs of depression (Dantzer and Kelley, 1989). In preclinical studies, treatment with anti-inflammatory agents attenuated depressive-like behavior in animals (Wang et al., 2018; Yue et al., 2018). With regard to anxiety disorders, elevated levels of TNF-α and IL-1β in the cerebrospinal fluid of relapsing-remitting multiple sclerosis patients were found to be predictive of poor performance on psychometric tests used to evaluate anxiety levels (Rossi et al., 2017). Blocking the activity of proinflammatory mediators or microglial activation via pharmacologic manipulation and genetic deletion of inflammatory components mitigated the development of stress-induced anxiety-like behavior in preclinical models (Wang et al., 2018; Wohleb et al., 2014; Yue et al., 2018).

Epilepsy is a neurological disease characterized by recurrent seizures. Over the past decade, a prominent role for glial cells in the mechanisms of seizure initiation and recurrence has been defined (Devinsky et al., 2013; Rana and Musto, 2018). Altered phenotype and function of activated astrocytes and microglial cells were detected in experimental and human epileptic tissue, including modifications in the induction of cytokines, chemokines, prostaglandins, complement factors, and cell adhesion molecules involved in both the pro- and anti-inflammatory components of neuroinflammation (Seifert and Steinhauser, 2013; Vezzani et al., 2013). In preclinical models of SE-induced epilepsy, which is thought to

be mechanistically similar to acquired epilepsy following OP-induced SE, inflammatory cytokines, particularly IL-1β and TNF-α, promoted the progression of epileptogenesis (Vezzani et al., 2013). Conversely, pharmacological attenuation of inflammation following SE with immunosuppressants or COX 2 inhibitors was efficacious in preventing epileptogenesis (Jung et al., 2006; Vezzani et al., 2013).

5.2 Neuroinflammation in OP neurotoxicity: Neurotoxic or neuroprotective?

The general assumption has been that OP-induced neuroinflammation contributes to the neuropathology and neurological deficits associated with neurotoxic OP exposures. This assumption is based on several lines of evidence. First, histopathological characterization of brains from rats that survived acute OP-induced SE have shown that neuroinflammatory responses typically coincide spatially and temporally with neurodegeneration (Ferchmin et al., 2014; Kim et al., 1999; Li et al., 2015; Liu et al., 2012; Rojas et al., 2015) and either precede or overlap with the onset of behavioral deficits (Coubard et al., 2008; Flannery et al., 2016; Rojas et al., 2015) and recurrent spontaneous seizures (De Araujo Furtado et al., 2010). Second, there is evidence that neuroinflammation exacerbates neuronal damage caused by excitotoxicity (Morimoto et al., 2002), in part via interactions between proinflammatory cytokines and glutamatergic neurotransmission (Fogal and Hewett, 2008). Specifically, IL-1β and TNF-α can hinder astrocytic uptake of synaptic glutamate and increase NMDA receptor activity, actions that promote excitotoxic neuronal cell death (Jander et al., 2000; Lawrence et al., 1998; Vezzani et al., 2013). These interactions are particularly important in the context of acute OP intoxication in which excessive cholinergic stimulation due to AChE inhibition increases glutamatergic signaling (Mcdonough and Shih, 1997). As shown in Fig. 2, excessive activation of NMDA receptors leads to elevated calcium influx into neurons, which perturbs mitochondria to increase ROS production. Excessive ROS ultimately results in apoptotic and necrotic cell death, which triggers neuroinflammatory responses, including increased levels of IL-1β and TNF-α, thereby setting up a feed-forward mechanism. Whether anti-inflammatory agents, particularly those that target IL-1β and TNF-α, can effectively decrease excitotoxic neurodegeneration following acute OP intoxication has yet to be systematically investigated.

The third line of evidence that neuroinflammation contributes to the neurotoxic outcomes of OP exposure are findings from a limited number of preclinical studies suggesting the potential therapeutic benefits of treating

OP intoxication with agents designed to block neuroinflammatory mediators. Most of these studies have been conducted in preclinical models of OP-induced SE. For example, a bifunctional molecule with a non-steroidal anti-inflammatory drug coupled to a pyridostigmine moiety increased survival rates and attenuated brain edema in mice acutely intoxicated with soman (Amitai et al., 2006). Treatment with the anti-inflammatory growth factor neuregulin-1 (NRG-1) inhibited microglial cell activation and reduced delayed neuronal cell death in multiple brain regions in a rat model of acute DFP intoxication (Li et al., 2012). A small molecule inhibitor of the prostaglandin E2 receptor, EP2, significantly decreased microglial activation, but not reactive astrogliosis, during the first few days following acute intoxication with DFP (Rojas et al., 2015). Furthermore, EP2 also attenuated the cognitive deficits in the novel object recognition task often seen following DFP (Rojas et al., 2016). There is at least one study that has tested the effects of anti-inflammatory treatment in a preclinical model of repeated low-level OP exposure. Dexamethasone and acetylsalicylic acid, when administered independently, improved locomotor performance and decreased degeneration of dopaminergic neurons in the substantia nigra of adult male mice exposed to neurotoxic levels of malathion (Ahmed et al., 2017).

While pharmacological studies with anti-inflammatory agents suggest that neuroinflammation contributes to the neurotoxic effects of OPs, there is evidence that neuroinflammatory responses may also function in repairing brain damage following OP-induced SE. In a mouse model of acute soman intoxication, it was observed that glial activation triggered the release of not only the proinflammatory cytokines, IL-6, TNF-α, and IL-1β, but also astrocytic secretion of neurotrophic and other growth factors that promote angiogenesis and neurogenesis (Collombet et al., 2011). These astrocyte-derived growth factors included ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF), fibroblast growth factor (FGF-2), nerve growth factor (NGF), and vascular endothelial growth factor (VEGF) (Collombet et al., 2011). Following acute intoxication with soman, neural progenitor cell proliferation in the subventricular zone and subgranular zone of the dentate gyrus was initially decreased (Collombet et al., 2005a). However, this transient reduction was followed by enhanced neural progenitor cell proliferation above normal levels a month after exposure. The timing of neurogenesis coincided with the onset of astroglial activation, while delayed neuronal death occurred when growth factor levels decreased, approximately 2–3 months after the initial OP exposure (Collombet et al., 2005a, 2007).

These observations suggest that reactive astrocytes may play an important role in neurorepair via secretion of neurotrophic and angiogenic growth

factors, and that increasing and/or prolonging the elevated expression of these factors may be of therapeutic benefit in treating acute OP intoxication. In support of this hypothesis, administration of FGF-2 and epidermal growth factor (EGF) to mice acutely intoxicated with soman significantly increased progenitor cells in the CA1 region of the hippocampus and amygdala 1 month after soman exposure (Collombet et al., 2005b). Subsequent studies demonstrated that FGF-2 and EGF co-administration accelerated the rate of neuronal regeneration in the hippocampus, and improved anxiety-like neurobehavioral profiles in soman-exposed mice, as measured by elevated plus maze and fear conditioning (Collombet et al., 2011). This co-treatment did not, however, alter neuronal regeneration in the amygdala, nor did it enhance the restoration of hippocampal-dependent memory-related tasks. Interestingly, administration of these growth factors did not alter the severity or duration of soman-induced seizures, nor did it decrease neuronal cell loss during the first 9 days after soman exposure. What is not known is whether endogenous growth factors released by activated astrocytes *in vivo* influence neuroregeneration and behavioral recovery following acute OP intoxication. Nonetheless, these data suggest that neuroinflammation may be functionally important in the regenerative processes that occur following acute OP intoxication. However, we are still far from a clear understanding of the spatiotemporal profile of inflammatory mediators that contribute to promoting damage as opposed to repair.

6. Critical data gaps and conclusions

Collectively, there is clear and compelling evidence that acute intoxication with OPs at levels that cause an acute and profound inhibition of AChE is associated with robust neuroinflammatory responses in preclinical rodent models (Table 2). While not as extensively studied, emerging data indicate that neuroinflammation is also present in preclinical models of repeated low-level OP exposures (Table 3). However, a key question that has yet to be addressed is whether neurotoxic exposures to OPs similarly elicit neuroinflammatory responses in humans. This critical data gap likely reflects, in part, the technical challenges of assessing neuroinflammation in the living human brain. *In vivo* imaging offers one solution to this challenge. PET imaging of TSPO, a molecular biomarker of neuroinflammation, is already used in the clinical setting (Guilarte, 2018), and has been validated in a preclinical model of acute OP intoxication (Flannery et al., 2016). The recent demonstration that high-order diffusion MRI was effective in

quantifying neuroinflammation in a rat model of OP-induced Gulf War Illness (Koo et al., 2018) suggests an alternate *in vivo* imaging approach for measuring neuroinflammation in OP-intoxicated humans. These *in vivo* imaging modalities will also increase the feasibility of performing OP intoxication studies in non-human primates because the same individual can be imaged longitudinally over time, and the ability to capture pre-exposure images enables each individual to serve as its own control.

The mechanism(s) by which OPs trigger neuroinflammation is another outstanding question. While there is compelling evidence from preclinical studies of Gulf War Illness to suggest that cholinergic mechanisms are not the principal mechanism of OP-induced neuroinflammation, data indicating that OPs may directly stimulate peripheral immune cells via cholinergic receptor activation at the immunological synapse suggest that the mechanism(s) by which OPs cause neuroinflammation may be context-specific. Answering this question is critical for gaining insight on novel therapeutic approaches for mitigating neurological damage post-exposure and for preventing brain injury pre-exposure. Elucidating cholinergic involvement in OP-induced neuroinflammation, particularly in the context of repeated low-level OPs exposures, also has important implications regarding the use of AChE inhibition as a point of departure for human health risk assessments.

Another generalization emerging from the literature is that the neuroinflammatory response varies depending on the OP exposure paradigm. Across all OP exposure paradigms, neuroinflammation is a dynamic response that changes over time, and the temporal pattern of the response varies across brain regions. These observations present significant challenges in terms of using neuroinflammatory biomarkers as diagnostic tools because the interpretation of the data may vary depending on the nature and timing of assessment. The available evidence also suggests that the role of neuroinflammation changes over time post-exposure. While the data on this topic are limited, it seems that the early, rapid neuroinflammatory response during the first hours to days post-exposure may be neurotoxic, while the delayed but persistent neuroinflammation that occurs weeks to months post-exposure may be important in neurorepair. A remaining critical data gap is the identification of specific cellular and molecular mechanisms that mediate the harmful vs. beneficial effects of neuroinflammation following OP exposure. Reports suggest that microglial activation, but not reactive astrocytosis, is linked to memory impairment observed in a rat model of acute DFP intoxication (Rojas et al., 2015, 2016), whereas reactive astrocytes have been linked to enhanced neurogenesis and accelerated functional

recovery of affective, but not cognitive, behavior in a mouse model of acute soman intoxication (Collombet et al., 2011). Whether neuroinflammation similarly plays a dual role in the context of repeated low-level OP exposure is not known. Regardless, the therapeutic challenge moving forward will be to determine how to modulate neuroinflammatory responses to attenuate harmful effects while sparing beneficial effects, as this type of modulatory strategy is likely to be more effective than simply suppressing neuroinflammation altogether.

References

Abolaji, A.O., Ojo, M., Afolabi, T.T., Arowoogun, M.D., Nwawolor, D., Farombi, E.O., 2017. Protective properties of 6-gingerol-rich fraction from Zingiber officinale (ginger) on chlorpyrifos-induced oxidative damage and inflammation in the brain, ovary and uterus of rats. Chem. Biol. Interact. 270, 15–23.

Ahmed, D., Abdel-Rahman, R.H., Salama, M., El-Zalabany, L.M., Mona Ahmed, E.-H., 2017. Malathion neurotoxic effects on dopaminergic system in mice: role of inflammation. J. Biomed. Sci. 6, 30–36.

Amitai, G., Adani, R., Fishbein, E., Meshulam, H., Laish, I., Dachir, S., 2006. Bifunctional compounds eliciting anti-inflammatory and anti-cholinesterase activity as potential treatment of nerve and blister chemical agents poisoning. J. Appl. Toxicol. 26, 81–87.

Angoa-Perez, M., Kreipke, C.W., Thomas, D.M., Van Shura, K.E., Lyman, M., Mcdonough, J.H., Kuhn, D.M., 2010. Soman increases neuronal COX-2 levels: possible link between seizures and protracted neuronal damage. Neurotoxicology 31, 738–746.

Arundine, M., Tymianski, M., 2003. Molecular mechanisms of calcium-dependent neurodegeneration in excitotoxicity. Cell Calcium 34, 325–337.

Astiz, M., Diz-Chaves, Y., Garcia-Segura, L.M., 2013. Sub-chronic exposure to the insecticide dimethoate induces a proinflammatory status and enhances the neuroinflammatory response to bacterial lypopolysaccharide in the hippocampus and striatum of male mice. Toxicol. Appl. Pharmacol. 272, 263–271.

Baille, V., Clarke, P.G., Brochier, G., Dorandeu, F., Verna, J.M., Four, E., Lallement, G., Carpentier, P., 2005. Soman-induced convulsions: the neuropathology revisited. Toxicology 215, 1–24.

Baille-Le Crom, V., Collombet, J.M., Carpentier, P., Brochier, G., Burckhart, M.F., Foquin, A., Pernot-Marino, I., Rondouin, G., Lallement, G., 1995. Early regional changes of GFAP mRNA in rat hippocampus and dentate gyrus during soman-induced seizures. Neuroreport 7, 365–369.

Balabanov, R., Strand, K., Goswami, R., Mcmahon, E., Begolka, W., Miller, S.D., Popko, B., 2007. Interferon-gamma-oligodendrocyte interactions in the regulation of experimental autoimmune encephalomyelitis. J. Neurosci. 27, 2013–2024.

Baltazar, M.T., Dinis-Oliveira, R.J., De Lourdes Bastos, M., Tsatsakis, A.M., Duarte, J.A., Carvalho, F., 2014. Pesticides exposure as etiological factors of Parkinson's disease and other neurodegenerative diseases—a mechanistic approach. Toxicol. Lett. 230, 85–103.

Banks, C.N., Lein, P.J., 2012. A review of experimental evidence linking neurotoxic organophosphorus compounds and inflammation. Neurotoxicology 33, 575–584.

Barr, D.B., Wong, L.Y., Bravo, R., Weerasekera, G., Odetokun, M., Restrepo, P., Kim, D.G., Fernandez, C., Whitehead Jr., R.D., Perez, J., Gallegos, M., Williams, B.L., Needham, L.L., 2011. Urinary concentrations of dialkylphosphate metabolites of organophosphorus pesticides: National health and nutrition examination survey 1999-2004. Int. J. Environ. Res. Public Health 8, 3063–3098.

Becher, B., Spath, S., Goverman, J., 2017. Cytokine networks in neuroinflammation. Nat. Rev. Immunol. 17, 49–59.
Beers, D.R., Henkel, J.S., Zhao, W., Wang, J., Appel, S.H., 2008. Cd4+ T cells support glial neuroprotection, slow disease progression, and modify glial morphology in an animal model of inherited ALS. Proc. Natl. Acad. Sci. U.S.A. 105, 15558–15563.
Binukumar, B.K., Bal, A., Gill, K.D., 2011. Chronic dichlorvos exposure: microglial activation, proinflammatory cytokines and damage to nigrostriatal dopaminergic system. NeuroMolecular Med. 13, 251–265.
Bird, S.B., Gaspari, R.J., Dickson, E.W., 2003. Early death due to severe organophosphate poisoning is a centrally mediated process. Acad. Emerg. Med. 10, 295–298.
Biswas, S.K., 2016. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? Oxidative Med. Cell. Longev. 2016, 5698931.
Bouchard, M.F., Chevrier, J., Harley, K.G., Kogut, K., Vedar, M., Calderon, N., Trujillo, C., Johnson, C., Bradman, A., Barr, D.B., Eskenazi, B., 2011. Prenatal exposure to organophosphate pesticides and IQ in 7-year-old children. Environ. Health Perspect. 119, 1189–1195.
Bsibsi, M., Ravid, R., Gveric, D., Van Noort, J.M., 2002. Broad expression of toll-like receptors in the human central nervous system. J. Neuropathol. Exp. Neurol. 61, 1013–1021.
Buckley, N.A., Eddleston, M., Li, Y., Bevan, M., Robertson, J., 2011. Oximes for acute organophosphate pesticide poisoning. Cochrane Database Syst. Rev. https://doi.org/10.1002/14651858.CD005085.pub2. CD005085.
Burke, R.D., Todd, S.W., Lumsden, E., Mullins, R.J., Mamczarz, J., Fawcett, W.P., Gullapalli, R.P., Randall, W.R., Pereira, E.F.R., Albuquerque, E.X., 2017. Developmental neurotoxicity of the organophosphorus insecticide chlorpyrifos: from clinical findings to preclinical models and potential mechanisms. J. Neurochem. 142 (Suppl. 2), 162–177.
Bushnell, P.J., Moser, V.C., 2006. Behavioral toxicity of cholinesterase inhibitors. In: Gupta, R.C. (Ed.), Toxicology of Organophosphate & Carbamate Compounds. Elsevier, San Diego, CA.
Chahine, L.M., Stern, M.B., Chen-Plotkin, A., 2014. Blood-based biomarkers for Parkinson's disease. Parkinsonism Relat. Disord. 20 (Suppl. 1), S99–103.
Chapman, S., Kadar, T., Gilat, E., 2006. Seizure duration following sarin exposure affects neuro-inflammatory markers in the rat brain. Neurotoxicology 27, 277–283.
Chen, Y., 2012. Organophosphate-induced brain damage: mechanisms, neuropsychiatric and neurological consequences, and potential therapeutic strategies. Neurotoxicology 33, 391–400.
Chung, H.Y., Sung, B., Jung, K.J., Zou, Y., Yu, B.P., 2006. The molecular inflammatory process in aging. Antioxid. Redox Signal. 8, 572–581.
Clemente, D., Ortega, M.C., Melero-Jerez, C., De Castro, F., 2013. The effect of glia-glia interactions on oligodendrocyte precursor cell biology during development and in demyelinating diseases. Front. Cell. Neurosci. 7, 268.
Clune, A.L., Ryan, P.B., Barr, D.B., 2012. Have regulatory efforts to reduce organophosphorus insecticide exposures been effective? Environ. Health Perspect. 120, 521–525.
Collombet, J.M., 2011. Nerve agent intoxication: recent neuropathophysiological findings and subsequent impact on medical management prospects. Toxicol. Appl. Pharmacol. 255, 229–241.
Collombet, J.M., Four, E., Bernabe, D., Masqueliez, C., Burckhart, M.F., Baille, V., Baubichon, D., Lallement, G., 2005a. Soman poisoning increases neural progenitor proliferation and induces long-term glial activation in mouse brain. Toxicology 208, 319–334.

Collombet, J.M., Four, E., Burckhart, M.F., Masqueliez, C., Bernabe, D., Baubichon, D., Herodin, F., Lallement, G., 2005b. Effect of cytokine treatment on the neurogenesis process in the brain of soman-poisoned mice. Toxicology 210, 9–23.
Collombet, J.M., Four, E., Fauquette, W., Burckhart, M.F., Masqueliez, C., Bernabe, D., Baubichon, D., Lallement, G., 2007. Soman poisoning induces delayed astrogliotic scar and angiogenesis in damaged mouse brain areas. Neurotoxicology 28, 38–48.
Collombet, J.M., Beracochea, D., Liscia, P., Pierard, C., Lallement, G., Filliat, P., 2011. Long-term effects of cytokine treatment on cognitive behavioral recovery and neuronal regeneration in soman-poisoned mice. Behav. Brain Res. 221, 261–270.
Corps, K.N., Roth, T.L., Mcgavern, D.B., 2015. Inflammation and neuroprotection in traumatic brain injury. JAMA Neurol. 72, 355–362.
Costa, L.G., 2006. Current issues in organophosphate toxicology. Clin. Chim. Acta 366, 1–13.
Costa, L.G., 2018. Organophosphorus compounds at 80: some old and new issues. Toxicol. Sci. 162, 24–35.
Coubard, S., Beracochea, D., Collombet, J.M., Philippin, J.N., Krazem, A., Liscia, P., Lallement, G., Pierard, C., 2008. Long-term consequences of soman poisoning in mice: part 2. Emotional behavior. Behav. Brain Res. 191, 95–103.
Dallerac, G., Rouach, N., 2016. Astrocytes as new targets to improve cognitive functions. Prog. Neurobiol. 144, 48–67.
Damodaran, T.V., Abou-Donia, M.B., 2000. Alterations in levels of mRNAs coding for glial fibrillary acidic protein (GFAP) and vimentin genes in the central nervous system of hens treated with diisopropyl phosphorofluoridate (DFP). Neurochem. Res. 25, 809–816.
Damodaran, T.V., Bilska, M.A., Rahman, A.A., Abou-Doni, M.B., 2002. Sarin causes early differential alteration and persistent overexpression in mRNAs coding for glial fibrillary acidic protein (GFAP) and vimentin genes in the central nervous system of rats. Neurochem. Res. 27, 407–415.
Damodaran, T.V., Patel, A.G., Greenfield, S.T., Dressman, H.K., Lin, S.M., Abou-Donia, M.B., 2006. Gene expression profiles of the rat brain both immediately and 3 months following acute sarin exposure. Biochem. Pharmacol. 71, 497–520.
Dantzer, R., Kelley, K.W., 1989. Stress and immunity: an integrated view of relationships between the brain and the immune system. Life Sci. 44, 1995–2008.
Dantzer, R., O'connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat. Rev. Neurosci. 9, 46–56.
Dantzer, R., O'connor, J.C., Lawson, M.A., Kelley, K.W., 2011. Inflammation-associated depression: from serotonin to kynurenine. Psychoneuroendocrinology 36, 426–436.
Davalos, D., Grutzendler, J., Yang, G., Kim, J.V., Zuo, Y., Jung, S., Littman, D.R., Dustin, M.L., Gan, W.B., 2005. ATP mediates rapid microglial response to local brain injury in vivo. Nat. Neurosci. 8, 752–758.
De Araujo Furtado, M., Lumley, L.A., Robison, C., Tong, L.C., Lichtenstein, S., Yourick, D.L., 2010. Spontaneous recurrent seizures after status epilepticus induced by soman in Sprague-Dawley rats. Epilepsia 51, 1503–1510.
De Araujo Furtado, M., Rossetti, F., Chanda, S., Yourick, D., 2012. Exposure to nerve agents: from status epilepticus to neuroinflammation, brain damage, neurogenesis and epilepsy. Neurotoxicology 33, 1476–1490.
Delgado, E., Mcconnell, R., Miranda, J., Keifer, M., Lundberg, I., Partanen, T., Wesseling, C., 2004. Central nervous system effects of acute organophosphate poisoning in a two-year follow-up. Scand. J. Work Environ. Health 30, 362–370.
Deshpande, L.S., Phillips, K., Huang, B., Delorenzo, R.J., 2014. Chronic behavioral and cognitive deficits in a rat survival model of paraoxon toxicity. Neurotoxicology 44, 352–357.

Devinsky, O., Vezzani, A., Najjar, S., De Lanerolle, N.C., Rogawski, M.A., 2013. Glia and epilepsy: excitability and inflammation. Trends Neurosci. 36, 174–184.
Dhote, F., Peinnequin, A., Carpentier, P., Baille, V., Delacour, C., Foquin, A., Lallement, G., Dorandeu, F., 2007. Prolonged inflammatory gene response following soman-induced seizures in mice. Toxicology 238, 166–176.
Dillman 3rd, J.F., Phillips, C.S., Kniffin, D.M., Tompkins, C.P., Hamilton, T.A., Kan, R.K., 2009. Gene expression profiling of rat hippocampus following exposure to the acetylcholinesterase inhibitor soman. Chem. Res. Toxicol. 22, 633–638.
Disabato, D.J., Quan, N., Godbout, J.P., 2016. Neuroinflammation: the devil is in the details. J. Neurochem. 139 (Suppl. 2), 136–153.
Dos Santos, A.A., Naime, A.A., De Oliveira, J., Colle, D., Dos Santos, D.B., Hort, M.A., Moreira, E.L., Sunol, C., De Bem, A.F., Farina, M., 2016. Long-term and low-dose malathion exposure causes cognitive impairment in adult mice: evidence of hippocampal mitochondrial dysfunction, astrogliosis and apoptotic events. Arch. Toxicol. 90, 647–660.
Duramad, P., Tager, I.B., Leikauf, J., Eskenazi, B., Holland, N.T., 2006. Expression of Th1/Th2 cytokines in human blood after in vitro treatment with chlorpyrifos, and its metabolites, in combination with endotoxin LPS and allergen Der p1. J. Appl. Toxicol. 26, 458–465.
Duysen, E.G., Li, B., Xie, W., Schopfer, L.M., Anderson, R.S., Broomfield, C.A., Lockridge, O., 2001. Evidence for nonacetylcholinesterase targets of organophosphorus nerve agent: supersensitivity of acetylcholinesterase knockout mouse to VX lethality. J. Pharmacol. Exp. Ther. 299, 528–535.
Dziedzic, T., 2006. Systemic inflammatory markers and risk of dementia. Am. J. Alzheimers Dis. Other Demen. 21, 258–262.
Eddleston, M., Buckley, N.A., Eyer, P., Dawson, A.H., 2008. Management of acute organophosphorus pesticide poisoning. Lancet 371, 597–607.
Engel, S.M., Wetmur, J., Chen, J., Zhu, C., Barr, D.B., Canfield, R.L., Wolff, M.S., 2011. Prenatal exposure to organophosphates, paraoxonase 1, and cognitive development in childhood. Environ. Health Perspect. 119, 1182–1188.
Ferchmin, P.A., Andino, M., Reyes Salaman, R., Alves, J., Velez-Roman, J., Cuadrado, B., Carrasco, M., Torres-Rivera, W., Segarra, A., Martins, A.H., Lee, J.E., Eterovic, V.A., 2014. 4R-cembranoid protects against diisopropylfluorophosphate-mediated neurodegeneration. Neurotoxicology 44, 80–90.
Filliat, P., Baubichon, D., Burckhart, M.F., Pernot-Marino, I., Foquin, A., Masqueliez, C., Perrichon, C., Carpentier, P., Lallement, G., 1999. Memory impairment after soman intoxication in rat: correlation with central neuropathology. Improvement with anticholinergic and antiglutamatergic therapeutics. Neurotoxicology 20, 535–549.
Filliat, P., Coubard, S., Pierard, C., Liscia, P., Beracochea, D., Four, E., Baubichon, D., Masqueliez, C., Lallement, G., Collombet, J.M., 2007. Long-term behavioral consequences of soman poisoning in mice. Neurotoxicology 28, 508–519.
Finkelstein, A., Kunis, G., Berkutzki, T., Ronen, A., Krivoy, A., Yoles, E., Last, D., Mardor, Y., Van Shura, K., Mcfarland, E., Capacio, B.A., Eisner, C., Gonzales, M., Gregorowicz, D., Eisenkraft, A., Mcdonough, J.H., Schwartz, M., 2012. Immunomodulation by poly-ye reduces organophosphate-induced brain damage. Brain Behav. Immun. 26, 159–169.
Flannery, B.M., Bruun, D.A., Rowland, D.J., Banks, C.N., Austin, A.T., Kukis, D.L., Li, Y., Ford, B.D., Tancredi, D.J., Silverman, J.L., Cherry, S.R., Lein, P.J., 2016. Persistent neuroinflammation and cognitive impairment in a rat model of acute diisopropylfluorophosphate intoxication. J. Neuroinflammation 13, 267.
Fogal, B., Hewett, S.J., 2008. Interleukin-1beta: a bridge between inflammation and excitotoxicity? J. Neurochem. 106, 1–23.

Frank-Cannon, T.C., Alto, L.T., Mcalpine, F.E., Tansey, M.G., 2009. Does neuroinflammation fan the flame in neurodegenerative diseases? Mol. Neurodegener. 4, 47.
Garcia, S.J., Seidler, F.J., Qiao, D., Slotkin, T.A., 2002. Chlorpyrifos targets developing glia: effects on glial fibrillary acidic protein. Brain Res. Dev. Brain Res. 133, 151–161.
Goldmann, T., Prinz, M., 2013. Role of microglia in CNS autoimmunity. Clin. Dev. Immunol. 2013, 208093.
Gomez-Gimenez, B., Llansola, M., Hernandez-Rabaza, V., Cabrera-Pastor, A., Malaguarnera, M., Agusti, A., Felipo, V., 2017. Sex-dependent effects of developmental exposure to different pesticides on spatial learning. The role of induced neuroinflammation in the hippocampus. Food Chem. Toxicol. 99, 135–148.
Grauer, E., Chapman, S., Rabinovitz, I., Raveh, L., Weissman, B.A., Kadar, T., Allon, N., 2008. Single whole-body exposure to sarin vapor in rats: long-term neuronal and behavioral deficits. Toxicol. Appl. Pharmacol. 227, 265–274.
Guedes, J.R., Lao, T., Cardoso, A.L., El Khoury, J., 2018. Roles of microglial and monocyte chemokines and their receptors in regulating Alzheimer's disease-associated amyloid-beta and tau pathologies. Front. Neurol. 9, 549.
Guilarte, T.R., 2018. TSPO in diverse CNS pathologies and psychiatric disease: a critical review and a way forward. Pharmacol. Ther. https://doi.org/10.1016/j.pharmthera.2018.09.003. S0163-7258(18)30157-8 [pii].
Gunnell, D., Eddleston, M., Phillips, M.R., Konradsen, F., 2007. The global distribution of fatal pesticide self-poisoning: systematic review. BMC Public Health 7, 357.
Henderson, R.F., Barr, E.B., Blackwell, W.B., Clark, C.R., Conn, C.A., Kalra, R., March, T.H., Sopori, M.L., Tesfaigzi, Y., Menache, M.G., Mash, D.C., 2002. Response of rats to low levels of sarin. Toxicol. Appl. Pharmacol. 184, 67–76.
Heneka, M.T., Carson, M.J., El Khoury, J., Landreth, G.E., Brosseron, F., Feinstein, D.L., Jacobs, A.H., Wyss-Coray, T., Vitorica, J., Ransohoff, R.M., Herrup, K., Frautschy, S.A., Finsen, B., Brown, G.C., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz, E., Halle, A., Petzold, G.C., Town, T., Morgan, D., Shinohara, M.L., Perry, V.H., Holmes, C., Bazan, N.G., Brooks, D.J., Hunot, S., Joseph, B., Deigendesch, N., Garaschuk, O., Boddeke, E., Dinarello, C.A., Breitner, J.C., Cole, G.M., Golenbock, D.T., Kummer, M.P., 2015. Neuroinflammation in Alzheimer's disease. Lancet Neurol. 14, 388–405.
Herman, F.J., Pasinetti, G.M., 2018. Principles of inflammasome priming and inhibition: implications for psychiatric disorders. Brain Behav. Immun. 73, 66–84.
Hobson, B.A., Siso, S., Rowland, D.J., Harvey, D.J., Bruun, D.A., Garbow, J.R., Lein, P.J., 2017. From the cover: magneticresonance imaging reveals progressive brain injury in rats acutely intoxicated with diisopropylfluorophosphate. Toxicol. Sci. 157, 342–353.
Hong, S., Dissing-Olesen, L., Stevens, B., 2016. New insights on the role of microglia in synaptic pruning in health and disease. Curr. Opin. Neurobiol. 36, 128–134.
Hulse, E.J., Davies, J.O., Simpson, A.J., Sciuto, A.M., Eddleston, M., 2014. Respiratory complications of organophosphorus nerve agent and insecticide poisoning. Implications for respiratory and critical care. Am. J. Respir. Crit. Care Med. 190, 1342–1354.
Jander, S., Schroeter, M., Stoll, G., 2000. Role of NMDA receptor signaling in the regulation of inflammatory gene expression after focal brain ischemia. J. Neuroimmunol. 109, 181–187.
Jensen, C.J., Massie, A., De Keyser, J., 2013. Immune players in the CNS: the astrocyte. J. Neuroimmune Pharmacol. 8, 824–839.
Jeon, S.W., Kim, Y.K., 2018. The role of neuroinflammation and neurovascular dysfunction in major depressive disorder. J. Inflamm. Res. 11, 179–192.
Johnson, E.A., Kan, R.K., 2010. The acute phase response and soman-induced status epilepticus: temporal, regional and cellular changes in rat brain cytokine concentrations. J. Neuroinflammation 7, 40.

Johnson, E.A., Dao, T.L., Guignet, M.A., Geddes, C.E., Koemeter-Cox, A.I., Kan, R.K., 2011. Increased expression of the chemokines CXCL1 and MIP-1alpha by resident brain cells precedes neutrophil infiltration in the brain following prolonged soman-induced status epilepticus in rats. J. Neuroinflammation 8, 41.
Joseph, J.A., Shukitt-Hale, B., Casadesus, G., 2005. Reversing the deleterious effects of aging on neuronal communication and behavior: beneficial properties of fruit polyphenolic compounds. Am. J. Clin. Nutr. 81, 313S–316S.
Jung, K.H., Chu, K., Lee, S.T., Kim, J., Sinn, D.I., Kim, J.M., Park, D.K., Lee, J.J., Kim, S.U., Kim, M., Lee, S.K., Roh, J.K., 2006. Cyclooxygenase-2 inhibitor, celecoxib, inhibits the altered hippocampal neurogenesis with attenuation of spontaneous recurrent seizures following pilocarpine-induced status epilepticus. Neurobiol. Dis. 23, 237–246.
Kennedy, K.A., Sandiford, S.D., Skerjanc, I.S., Li, S.S., 2012. Reactive oxygen species and the neuronal fate. Cell. Mol. Life Sci. 69, 215–221.
Kim, Y.K., Jeon, S.W., 2018. Neuroinflammation and the immune-kynurenine pathway in anxiety disorders. Curr. Neuropharmacol. 16, 574–582.
Kim, Y.B., Hur, G.H., Shin, S., Sok, D.E., Kang, J.K., Lee, Y.S., 1999. Organophosphate-induced brain injuries: delayed apoptosis mediated by nitric oxide. Environ. Toxicol. Pharmacol. 7, 147–152.
Kimelberg, H.K., Nedergaard, M., 2010. Functions of astrocytes and their potential as therapeutic targets. Neurotherapeutics 7, 338–353.
Kleen, J.K., Scott, R.C., Lenck-Santini, P.P., Holmes, G.L., 2012. Cognitive and behavioral co-morbidities of epilepsy. In: Noebels, J.L., Avoli, M., Rogawski, M.A., Olsen, R.W., Delgado-Escueta, A.V. (Eds.), Jasper's Basic Mechanisms of the Epilepsies, fourth ed, Bethesda, MD.
Konishi, H., Kiyama, H., 2018. Microglial TREM2/DAP12 signaling: a double-edged sword in neural diseases. Front. Cell. Neurosci. 12, 206.
Koo, B.B., Michalovicz, L.T., Calderazzo, S., Kelly, K.A., Sullivan, K., Killiany, R.J., O'callaghan, J.P., 2018. Corticosterone potentiates DFP-induced neuroinflammation and affects high-order diffusion imaging in a rat model of gulf war illness. Brain Behav. Immun. 67, 42–46.
Kraft, A.D., Harry, G.J., 2011. Features of microglia and neuroinflammation relevant to environmental exposure and neurotoxicity. Int. J. Environ. Res. Public Health 8, 2980–3018.
Kuruba, R., Wu, X., Reddy, D.S., 2018. Benzodiazepine-refractory status epilepticus, neuroinflammation, and interneuron neurodegeneration after acute organophosphate intoxication. Biochim. Biophys. Acta 1864, 2845–2858.
Lavisse, S., Guillermier, M., Herard, A.S., Petit, F., Delahaye, M., Van Camp, N., Ben Haim, L., Lebon, V., Remy, P., Dolle, F., Delzescaux, T., Bonvento, G., Hantraye, P., Escartin, C., 2012. Reactive astrocytes overexpress TSPO and are detected by TSPO positron emission tomography imaging. J. Neurosci. 32, 10809–10818.
Lawrence, C.B., Allan, S.M., Rothwell, N.J., 1998. Interleukin-1beta and the interleukin-1 receptor antagonist act in the striatum to modify excitotoxic brain damage in the rat. Eur. J. Neurosci. 10, 1188–1195.
Lemercier, G., Carpentier, P., Sentenac-Roumanou, H., Morelis, P., 1983. Histological and histochemical changes in the central nervous system of the rat poisoned by an irreversible anticholinesterase organophosphorus compound. Acta Neuropathol. 61, 123–129.
Levin, H.S., Rodnitzky, R.L., Mick, D.L., 1976. Anxiety associated with exposure to organophosphate compounds. Arch. Gen. Psychiatry 33, 225–228.
Li, Y., Lein, P.J., Liu, C., Bruun, D.A., Tewolde, T., Ford, G., Ford, B.D., 2011. Spatiotemporal pattern of neuronal injury induced by DFP in rats: a model for delayed neuronal cell death following acute op intoxication. Toxicol. Appl. Pharmacol. 253, 261–269.

Li, Y., Lein, P.J., Liu, C., Bruun, D.A., Giulivi, C., Ford, G.D., Tewolde, T., Ross-Inta, C., Ford, B.D., 2012. Neuregulin-1 is neuroprotective in a rat model of organophosphate-induced delayed neuronal injury. Toxicol. Appl. Pharmacol. 262, 194–204.

Li, Y., Lein, P.J., Ford, G.D., Liu, C., Stovall, K.C., White, T.E., Bruun, D.A., Tewolde, T., Gates, A.S., Distel, T.J., Surles-Zeigler, M.C., Ford, B.D., 2015. Neuregulin-1 inhibits neuroinflammatory responses in a rat model of organophosphate-nerve agent-induced delayed neuronal injury. J. Neuroinflammation 12, 64.

Liang, L.P., Pearson-Smith, J.N., Huang, J., Mcelroy, P., Day, B.J., Patel, M., 2018. Neuroprotective effects of AEOL10150 in a rat organophosphate model. Toxicol. Sci. 162, 611–621.

Liedtke, W., Edelmann, W., Bieri, P.L., Chiu, F.C., Cowan, N.J., Kucherlapati, R., Raine, C.S., 1996. GFAP is necessary for the integrity of CNS white matter architecture and long-term maintenance of myelination. Neuron 17, 607–615.

Lim, K.L., Tay, A., Nadarajah, V.D., Mitra, N.K., 2011. The effect of consequent exposure of stress and dermal application of low doses of chlorpyrifos on the expression of glial fibrillary acidic protein in the hippocampus of adult mice. J. Occup. Med. Toxicol. 6, 4.

Liu, M., Bing, G., 2011. Lipopolysaccharide animal models for Parkinson's disease. Parkinsons Dis. 2011, 327089.

Liu, C., Li, Y., Lein, P.J., Ford, B.D., 2012. Spatiotemporal patterns of GFAP upregulation in rat brain following acute intoxication with diisopropylfluorophosphate (DFP). Curr. Neurobiol. 3, 90–97.

Loane, D.J., Kumar, A., Stoica, B.A., Cabatbat, R., Faden, A.I., 2014. Progressive neurodegeneration after experimental brain trauma: association with chronic microglial activation. J. Neuropathol. Exp. Neurol. 73, 14–29.

Locker, A.R., Michalovicz, L.T., Kelly, K.A., Miller, J.V., Miller, D.B., O'callaghan, J.P., 2017. Corticosterone primes the neuroinflammatory response to gulf war illness-relevant organophosphates independently of acetylcholinesterase inhibition. J. Neurochem. 142, 444–455.

Massaad, C.A., Klann, E., 2011. Reactive oxygen species in the regulation of synaptic plasticity and memory. Antioxid. Redox Signal. 14, 2013–2054.

Mcafoose, J., Baune, B.T., 2009. Evidence for a cytokine model of cognitive function. Neurosci. Biobehav. Rev. 33, 355–366.

Mcdonough Jr., J.H., Shih, T.M., 1997. Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology. Neurosci. Biobehav. Rev. 21, 559–579.

Mense, S.M., Sengupta, A., Lan, C., Zhou, M., Bentsman, G., Volsky, D.J., Whyatt, R.M., Perera, F.P., Zhang, L., 2006. The common insecticides cyfluthrin and chlorpyrifos alter the expression of a subset of genes with diverse functions in primary human astrocytes. Toxicol. Sci. 93, 125–135.

Mew, E.J., Padmanathan, P., Konradsen, F., Eddleston, M., Chang, S.S., Phillips, M.R., Gunnell, D., 2017. The global burden of fatal self-poisoning with pesticides 2006-15: systematic review. J. Affect. Disord. 219, 93–104.

Meyer-Baron, M., Knapp, G., Schaper, M., Van Thriel, C., 2015. Meta-analysis on occupational exposure to pesticides—neurobehavioral impact and dose-response relationships. Environ. Res. 136, 234–245.

Milatovic, D., Gupta, R.C., Aschner, M., 2006. Anticholinesterase toxicity and oxidative stress. ScientificWorldJournal 6, 295–310.

Miller, J.V., Lebouf, R.F., Kelly, K.A., Michalovicz, L.T., Ranpara, A., Locker, A.R., Miller, D.B., O'callaghan, J.P., 2018. The neuroinflammatory phenotype in a mouse model of gulf war illness is unrelated to brain regional levels of acetylcholine as measured by quantitative hilic-uplc-ms/ms. Toxicol. Sci. 165, 302–313. https://doi.org/10.1093/toxsci/kfy130.

Mitra, A., Sarkar, M., Chatterjee, C., 2017. Modulation of immune response by organophosphate pesticides: mammals as potential model. Proc. Zool. Soc. https://doi.org/10.1007/s12595-017-0256-5.

Miyaki, K., Nishiwaki, Y., Maekawa, K., Ogawa, Y., Asukai, N., Yoshimura, K., Etoh, N., Matsumoto, Y., Kikuchi, Y., Kumagai, N., Omae, K., 2005. Effects of sarin on the nervous system of subway workers seven years after the Tokyo subway sarin attack. J. Occup. Health 47, 299–304.

Mohammadzadeh, L., Hosseinzadeh, H., Abnous, K., Razavi, B.M., 2018. Neuroprotective potential of crocin against malathion-induced motor deficit and neurochemical alterations in rats. Environ. Sci. Pollut. Res. Int. 25, 4904–4914.

Morimoto, K., Murasugi, T., Oda, T., 2002. Acute neuroinflammation exacerbates excitotoxicity in rat hippocampus in vivo. Exp. Neurol. 177, 95–104.

Mosser, C.A., Baptista, S., Arnoux, I., Audinat, E., 2017. Microglia in CNS development: shaping the brain for the future. Prog. Neurobiol. 149–150, 1–20.

Munoz-Quezada, M.T., Lucero, B.A., Barr, D.B., Steenland, K., Levy, K., Ryan, P.B., Iglesias, V., Alvarado, S., Concha, C., Rojas, E., Vega, C., 2013. Neurodevelopmental effects in children associated with exposure to organophosphate pesticides: a systematic review. Neurotoxicology 39, 158–168.

Myhrer, T., Andersen, J.M., Nguyen, N.H., Aas, P., 2005. Soman-induced convulsions in rats terminated with pharmacological agents after 45 min: neuropathology and cognitive performance. Neurotoxicology 26, 39–48.

Naughton, S.X., Terry Jr., A.V., 2018. Neurotoxicity in acute and repeated organophosphate exposure. Toxicology 408, 101–112. https://doi.org/10.1016/j.tox.2018.08.011.

Nimmerjahn, A., Kirchhoff, F., Helmchen, F., 2005. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science 308, 1314–1318.

Nishiwaki, Y., Maekawa, K., Ogawa, Y., Asukai, N., Minami, M., Omae, K., Sarin Health Effects Study Group, 2001. Effects of sarin on the nervous system in rescue team staff members and police officers 3 years after the Tokyo subway sarin attack. Environ. Health Perspect. 109, 1169–1173.

O'callaghan, J.P., Kelly, K.A., Locker, A.R., Miller, D.B., Lasley, S.M., 2015. Corticosterone primes the neuroinflammatory response to DFP in mice: potential animal model of gulf war illness. J. Neurochem. 133, 708–721.

Okumura, T., Hisaoka, T., Naito, T., Isonuma, H., Okumura, S., Miura, K., Maekawa, H., Ishimatsu, S., Takasu, N., Suzuki, K., 2005. Acute and chronic effects of sarin exposure from the Tokyo subway incident. Environ. Toxicol. Pharmacol. 19, 447–450.

Olmos-Alonso, A., Schetters, S.T.T., Sri, S., Askew, K., Mancuso, R., Vargas-Caballero, M., Holscher, C., Perry, V.H., Gomez-Nicola, D., 2016. Pharmacological targeting of CSF1R inhibits microglial proliferation and prevents the progression of Alzheimer's-like pathology. Brain 139, 1–17. https://doi.org/10.1093/brain/awv379.

Pascual, O., Ben, Achour S., Rostaing, P., Triller, A., Bessis, A., 2012. Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. Proc. Natl. Acad. Sci. U.S.A. 109, E197–E205.

Pearson, J.N., Patel, M., 2016. The role of oxidative stress in organophosphate and nerve agent toxicity. Ann. N. Y. Acad. Sci. 1378, 17–24.

Peferoen, L., Kipp, M., Van Der Valk, P., Van Noort, J.M., Amor, S., 2014. Oligodendrocyte-microglia cross-talk in the central nervous system. Immunology 141, 302–313.

Pekny, M., Pekna, M., Messing, A., Steinhauser, C., Lee, J.M., Parpura, V., Hol, E.M., Sofroniew, M.V., Verkhratsky, A., 2016. Astrocytes: a central element in neurological diseases. Acta Neuropathol. 131, 323–345.

Pereira, E.F., Aracava, Y., Detolla Jr., L.J., Beecham, E.J., Basinger Jr., G.W., Wakayama, E.J., Albuquerque, E.X., 2014. Animal models that best reproduce the

clinical manifestations of human intoxication with organophosphorus compounds. J. Pharmacol. Exp. Ther. 350, 313–321.
Pfau, M.L., Russo, S.J., 2016. Neuroinflammation regulates cognitive impairment in socially defeated mice. Trends Neurosci. 39, 353–355.
Pfau, M.L., Menard, C., Russo, S.J., 2018. Inflammatory mediators in mood disorders: therapeutic opportunities. Annu. Rev. Pharmacol. Toxicol. 58, 411–428.
Pope, C.N., 1999. Organophosphorus pesticides: do they all have the same mechanism of toxicity? J. Toxicol. Environ. Health B Crit. Rev. 2, 161–181.
Potts, M.B., Koh, S.E., Whetstone, W.D., Walker, B.A., Yoneyama, T., Claus, C.P., Manvelyan, H.M., Noble-Haeusslein, L.J., 2006. Traumatic injury to the immature brain: inflammation, oxidative injury, and iron-mediated damage as potential therapeutic targets. NeuroRx 3, 143–153.
Prager, E.M., Aroniadou-Anderjaska, V., Almeida-Suhett, C.P., Figueiredo, T.H., Apland, J.P., Rossetti, F., Olsen, C.H., Braga, M.F., 2014a. The recovery of acetylcholinesterase activity and the progression of neuropathological and pathophysiological alterations in the rat basolateral amygdala after soman-induced status epilepticus: relation to anxiety-like behavior. Neuropharmacology 81, 64–74.
Prager, E.M., Pidoplichko, V.I., Aroniadou-Anderjaska, V., Apland, J.P., Braga, M.F., 2014b. Pathophysiological mechanisms underlying increased anxiety after soman exposure: reduced gabaergic inhibition in the basolateral amygdala. Neurotoxicology 44, 335–343.
Prinz, M., Priller, J., 2017. The role of peripheral immune cells in the CNS in steady state and disease. Nat. Neurosci. 20, 136–144.
Proskocil, B.J., Bruun, D.A., Jacoby, D.B., Van Rooijen, N., Lein, P.J., Fryer, A.D., 2013. Macrophage TNF-alpha mediates parathion-induced airway hyperreactivity in Guinea pigs. Am. J. Physiol. Lung Cell. Mol. Physiol. 304, L519–L529.
Raffaele, K., Hughey, D., Wenk, G., Olton, D., Modrow, H., Mcdonough, J., 1987. Long-term behavioral changes in rats following organophosphonate exposure. Pharmacol. Biochem. Behav. 27, 407–412.
Rahman, I., Biswas, S.K., Kirkham, P.A., 2006. Regulation of inflammation and redox signaling by dietary polyphenols. Biochem. Pharmacol. 72, 1439–1452.
Ramesh, G., Benge, S., Pahar, B., Philipp, M.T., 2012. A possible role for inflammation in mediating apoptosis of oligodendrocytes as induced by the Lyme disease spirochete Borrelia burgdorferi. J. Neuroinflammation 9, 72.
Rana, A., Musto, A.E., 2018. The role of inflammation in the development of epilepsy. J. Neuroinflammation 15, 144.
Ray, A., Liu, J., Ayoubi, P., Pope, C., 2010. Dose-related gene expression changes in forebrain following acute, low-level chlorpyrifos exposure in neonatal rats. Toxicol. Appl. Pharmacol. 248, 144–155.
Robel, S., Buckingham, S.C., Boni, J.L., Campbell, S.L., Danbolt, N.C., Riedemann, T., Sutor, B., Sontheimer, H., 2015. Reactive astrogliosis causes the development of spontaneous seizures. J. Neurosci. 35, 3330–3345.
Rohlman, D.S., Anger, W.K., Lein, P.J., 2011. Correlating neurobehavioral performance with biomarkers of organophosphorous pesticide exposure. Neurotoxicology 32, 268–276.
Rojas, A., Ganesh, T., Lelutiu, N., Gueorguieva, P., Dingledine, R., 2015. Inhibition of the prostaglandin ep2 receptor is neuroprotective and accelerates functional recovery in a rat model of organophosphorus induced status epilepticus. Neuropharmacology 93, 15–27.
Rojas, A., Ganesh, T., Manji, Z., O'neill, T., Dingledine, R., 2016. Inhibition of the prostaglandin E2 receptor EP2 prevents status epilepticus-induced deficits in the novel object recognition task in rats. Neuropharmacology 110, 419–430.

Rossi, S., Studer, V., Motta, C., Polidoro, S., Perugini, J., Macchiarulo, G., Giovannetti, A.M., Pareja-Gutierrez, L., Calo, A., Colonna, I., Furlan, R., Martino, G., Centonze, D., 2017. Neuroinflammation drives anxiety and depression in relapsing-remitting multiple sclerosis. Neurology 89, 1338–1347.

Sagiv, S.K., Harris, M.H., Gunier, R.B., Kogut, K.R., Harley, K.G., Deardorff, J., Bradman, A., Holland, N., Eskenazi, B., 2018a. Prenatal organophosphate pesticide exposure and traits related to autism spectrum disorders in a population living in proximity to agriculture. Environ. Health Perspect. 126, 047012.

Sagiv, S.K., Harris, M.H., Gunier, R.B., Kogut, K.R., Harley, K.G., Deardorff, J., Bradman, A., Holland, N., Eskenazi, B., 2018b. Erratum: "prenatal organophosphate pesticide exposure and traits related to autism spectrum disorders in a population living in proximity to agriculture". Environ. Health Perspect. 126, 079001.

Saijo, K., Glass, C.K., 2011. Microglial cell origin and phenotypes in health and disease. Nat. Rev. Immunol. 11, 775–787.

Sanchez-Santed, F., Colomina, M.T., Herrero Hernandez, E., 2016. Organophosphate pesticide exposure and neurodegeneration. Cortex 74, 417–426.

Savage, E.P., Keefe, T.J., Mounce, L.M., Heaton, R.K., Lewis, J.A., Burcar, P.J., 1988. Chronic neurological sequelae of acute organophosphate pesticide poisoning. Arch. Environ. Health 43, 38–45.

Schwartz, M., Shaked, I., Fisher, J., Mizrahi, T., Schori, H., 2003. Protective autoimmunity against the enemy within: fighting glutamate toxicity. Trends Neurosci. 26, 297–302.

Seifert, G., Steinhauser, C., 2013. Neuron-astrocyte signaling and epilepsy. Exp. Neurol. 244, 4–10.

Shaked, I., Porat, Z., Gersner, R., Kipnis, J., Schwartz, M., 2004. Early activation of microglia as antigen-presenting cells correlates with t cell-mediated protection and repair of the injured central nervous system. J. Neuroimmunol. 146, 84–93.

Shi, Y., Holtzman, D.M., 2018. Interplay between innate immunity and Alzheimer disease: APOE and TREM2 in the spotlight. Nat. Rev. Immunol. https://doi.org/10.1038/s41577-018-0051-1.

Shrot, S., Ramaty, E., Biala, Y., Bar-Klein, G., Daninos, M., Kamintsky, L., Makarovsky, I., Statlender, L., Rosman, Y., Krivoy, A., Lavon, O., Kassirer, M., Friedman, A., Yaari, Y., 2014. Prevention of organophosphate-induced chronic epilepsy by early benzodiazepine treatment. Toxicology 323, 19–25.

Simen, A.A., Bordner, K.A., Martin, M.P., Moy, L.A., Barry, L.C., 2011. Cognitive dysfunction with aging and the role of inflammation. Ther. Adv. Chronic Dis. 2, 175–195.

Siso, S., Hobson, B.A., Harvey, D.J., Bruun, D.A., Rowland, D.J., Garbow, J.R., Lein, P.J., 2017. Editor's highlight: spatiotemporal progression and remission of lesions in the rat brain following acute intoxication with diisopropylfluorophosphate. Toxicol. Sci. 157, 330–341.

Skaper, S.D., Facci, L., Zusso, M., Giusti, P., 2018. An inflammation-centric view of neurological disease: beyond the neuron. Front. Cell. Neurosci. 12, 72.

Soltaninejad, K., Abdollahi, M., 2009. Current opinion on the science of organophosphate pesticides and toxic stress: a systematic review. Med. Sci. Monit. 15, RA75–90.

Spradling, K.D., Lumley, L.A., Robison, C.L., Meyerhoff, J.L., Dillman 3rd, J.F., 2011a. Transcriptional analysis of rat piriform cortex following exposure to the organophosphonate anticholinesterase sarin and induction of seizures. J. Neuroinflammation 8, 83.

Spradling, K.D., Lumley, L.A., Robison, C.L., Meyerhoff, J.L., Dillman 3rd, J.F., 2011b. Transcriptional responses of the nerve agent-sensitive brain regions amygdala, hippocampus, piriform cortex, septum, and thalamus following exposure to the organophosphonate anticholinesterase sarin. J. Neuroinflammation 8, 84.

Stapleton, A.R., Chan, V.T., 2009. Subtoxic chlorpyrifos treatment resulted in differential expression of genes implicated in neurological functions and development. Arch. Toxicol. 83, 319–333.
Svensson, I., Waara, L., Johansson, L., Bucht, A., Cassel, G., 2001. Soman-induced interleukin-1 beta mRNA and protein in rat brain. Neurotoxicology 22, 355–362.
Thorburne, S.K., Juurlink, B.H., 1996. Low glutathione and high iron govern the susceptibility of oligodendroglial precursors to oxidative stress. J. Neurochem. 67, 1014–1022.
Tian, J., Dai, H., Deng, Y., Zhang, J., Li, Y., Zhou, J., Zhao, M., Zhao, M., Zhang, C., Zhang, Y., Wang, P., Bing, G., Zhao, L., 2015. The effect of HMGB1 on sub-toxic chlorpyrifos exposure-induced neuroinflammation in amygdala of neonatal rats. Toxicology 338, 95–103.
Turtzo, L.C., Lescher, J., Janes, L., Dean, D.D., Budde, M.D., Frank, J.A., 2014. Macrophagic and microglial responses after focal traumatic brain injury in the female rat. J. Neuroinflammation 11, 82.
Veiga, S., Carrero, P., Pernia, O., Azcoitia, I., Garcia-Segura, L.M., 2007. Translocator protein 18 kDa is involved in the regulation of reactive gliosis. Glia 55, 1426–1436.
Verma, S.K., Raheja, G., Gill, K.D., 2009. Role of muscarinic signal transduction and CREB phosphorylation in dichlorvos-induced memory deficits in rats: an acetylcholine independent mechanism. Toxicology 256, 175–182.
Vezzani, A., Moneta, D., Conti, M., Richichi, C., Ravizza, T., De Luigi, A., de Simoni, M.G., Sperk, G., Andell-Jonsson, S., Lundkvist, J., Iverfeldt, K., Bartfai, T., 2000. Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. Proc. Natl. Acad. Sci. U. S. A. 97, 11534–11539.
Vezzani, A., Aronica, E., Mazarati, A., Pittman, Q.J., 2013. Epilepsy and brain inflammation. Exp. Neurol. 244, 11–21.
Viviani, B., Boraso, M., Marchetti, N., Marinovich, M., 2014. Perspectives on neuroinflammation and excitotoxicity: a neurotoxic conspiracy? Neurotoxicology 43, 10–20.
Voorhees, J.R., Rohlman, D.S., Lein, P.J., Pieper, A.A., 2016. Neurotoxicity in preclinical models of occupational exposure to organophosphorus compounds. Front. Neurosci. 10, 590.
Wahner, A.D., Bronstein, J.M., Bordelon, Y.M., Ritz, B., 2007a. Nonsteroidal anti-inflammatory drugs may protect against Parkinson disease. Neurology 69, 1836–1842.
Wahner, A.D., Sinsheimer, J.S., Bronstein, J.M., Ritz, B., 2007b. Inflammatory cytokine gene polymorphisms and increased risk of Parkinson disease. Arch. Neurol. 64, 836–840.
Walker, K.R., Tesco, G., 2013. Molecular mechanisms of cognitive dysfunction following traumatic brain injury. Front. Aging Neurosci. 5, 29.
Wang, J.Y., Wen, L.L., Huang, Y.N., Chen, Y.T., Ku, M.C., 2006. Dual effects of antioxidants in neurodegeneration: direct neuroprotection against oxidative stress and indirect protection via suppression of glia-mediated inflammation. Curr. Pharm. Des. 12, 3521–3533.
Wang, G., Zhang, J., Hu, X., Zhang, L., Mao, L., Jiang, X., Liou, A.K., Leak, R.K., Gao, Y., Chen, J., 2013. Microglia/macrophage polarization dynamics in white matter after traumatic brain injury. J. Cereb. Blood Flow Metab. 33, 1864–1874.
Wang, Y.L., Han, Q.Q., Gong, W.Q., Pan, D.H., Wang, L.Z., Hu, W., Yang, M., Li, B., Yu, J., Liu, Q., 2018. Microglial activation mediates chronic mild stress-induced depressive- and anxiety-like behavior in adult rats. J. Neuroinflammation 15, 21.
Williams, K., Ulvestad, E., Waage, A., Antel, J.P., Mclaurin, J., 1994. Activation of adult human derived microglia by myelin phagocytosis in vitro. J. Neurosci. Res. 38, 433–443.
Williams, A.J., Berti, R., Yao, C., Price, R.A., Velarde, L.C., Koplovitz, I., Schultz, S.M., Tortella, F.C., Dave, J.R., 2003. Central neuro-inflammatory gene response following soman exposure in the rat. Neurosci. Lett. 349, 147–150.

Wohleb, E.S., Patterson, J.M., Sharma, V., Quan, N., Godbout, J.P., Sheridan, J.F., 2014. Knockdown of interleukin-1 receptor type-1 on endothelial cells attenuated stress-induced neuroinflammation and prevented anxiety-like behavior. J. Neurosci. 34, 2583–2591.

Wright, L.K., Liu, J., Nallapaneni, A., Pope, C.N., 2010. Behavioral sequelae following acute diisopropylfluorophosphate intoxication in rats: comparative effects of atropine and cannabinomimetics. Neurotoxicol. Teratol. 32, 329–335.

Wyss-Coray, T., Rogers, J., 2012. Inflammation in Alzheimer disease—a brief review of the basic science and clinical literature. Cold Spring Harb. Perspect. Med. 2, a006346.

Yanagisawa, N., Morita, H., Nakajima, T., 2006. Sarin experiences in Japan: acute toxicity and long-term effects. J. Neurol. Sci. 249, 76–85.

Yue, N., Li, B., Yang, L., Han, Q.Q., Huang, H.J., Wang, Y.L., Wang, J., Yu, R., Wu, G.C., Liu, Q., Yu, J., 2018. Electro-acupuncture alleviates chronic unpredictable stress-induced depressive- and anxiety-like behavior and hippocampal neuroinflammation in rat model of depression. Front. Mol. Neurosci. 11, 149.

Zaja-Milatovic, S., Gupta, R.C., Aschner, M., Milatovic, D., 2009. Protection of DFP-induced oxidative damage and neurodegeneration by antioxidants and NMDA receptor antagonist. Toxicol. Appl. Pharmacol. 240, 124–131.

Zajicek, J., Wing, M., Skepper, J., Compston, A., 1995. Human oligodendrocytes are not sensitive to complement. A study of CD59 expression in the human central nervous system. Lab. Investig. 73, 128–138.

Zhang, B., Gaiteri, C., Bodea, L.G., Wang, Z., Mcelwee, J., Podtelezhnikov, A.A., Zhang, C., Xie, T., Tran, L., Dobrin, R., Fluder, E., Clurman, B., Melquist, S., Narayanan, M., Suver, C., Shah, H., Mahajan, M., Gillis, T., Mysore, J., Macdonald, M.E., Lamb, J.R., Bennett, D.A., Molony, C., Stone, D.J., Gudnason, V., Myers, A.J., Schadt, E.E., Neumann, H., Zhu, J., Emilsson, V., 2013. Integrated systems approach identifies genetic nodes and networks in late-onset Alzheimer's disease. Cell 153, 707–720.

Zhou, H., Lapointe, B.M., Clark, S.R., Zbytnuik, L., Kubes, P., 2006. A requirement for microglial TLR4 in leukocyte recruitment into brain in response to lipopolysaccharide. J. Immunol. 177, 8103–8110.

Zimmer, L.A., Ennis, M., Shipley, M.T., 1997. Soman-induced seizures rapidly activate astrocytes and microglia in discrete brain regions. J. Comp. Neurol. 378, 482–492.