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Chemical Warfare Nerve Agent-Induced Effects on Gene Expression in Human Neuron and Astrocyte Cultures

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Abstract[1]

Acute exposure to organophosphorus nerve agents such as soman and VX cause neuronal degeneration, astrocytic activation and cell death in specific brain regions. Although *in vivo* neuronal cell death is readily observed after agent exposure, cell death has been difficult to observe in cultured neurons. Reports of VX-exposed primary rat neuron cultures indicate attenuation of cell death by N-methyl-D-aspartate receptor antagonism, but not by acetylcholinesterase activity protection or muscarinic receptor antagonism. To further investigate direct molecular effects, we examined gene expression changes induced by nerve agent exposure in primary cultures of human neurons and astrocytes. Cultured human astrocytes and neurons were exposed to soman or VX and harvested 24 h later for RNA isolation and microarray processing. Principal component analysis indicated neuronal gene expression changes in response to soman, but not VX exposure. Neuronal genes differentially expressed in response to soman exposure mapped to canonical pathways containing inflammatory molecules. Functional analysis of the soman-exposed neuronal dataset identified neurological disease and inflammatory response. In addition, gene networks associated with significant neurological disease function and inflammatory response demonstrate overlapping involvement of several transcripts, including *TNFRSF1A* and *IL1R1*. These data suggest a non-cholinergic effect of soman that induces transcriptional expression of inflammatory molecules.

[1] Abbreviations: 2-PAM, pyridine-2-aldoxime-methylchloride or pralidoxime; IPA, ingenuity pathway analysis; PCA, principal component analysis

Introduction

Exposure to organophosphorus compounds, such as the nerve agents soman and VX, can cause a progression of symptoms including hypersecretion, loss of consciousness, flaccid paralysis, seizure, and even death (Shih et al., 1991). These nerve agents inhibit acetylcholinesterase, resulting in increased acetylcholine concentrations in central synapses which in turn can trigger seizure activity in susceptible brain regions. Once initiated by these agents, sustained seizure activity leads to the release of excess glutamate, leading to glutamate-induced excitotoxicity and neuronal death (Solberg and Belkin, 1997). Loss of neuronal function can cause profound cognitive and behavioral changes that can drastically reduce quality of life for exposed survivors (Yanagisawa et al., 2006). Current medical countermeasures for nerve agent exposure consist of pretreatment with a reversible carbamate cholinesterase inhibitor (e.g., pyridostigmine) and immediate therapeutic treatment with an anticholinergic drug (e.g., atropine) and an oxime (e.g. pyridine-2-aldoxime methylchloride, or 2-PAM). In cases of severe intoxication, diazepam is administered to curb seizure activity. However, efficacy of diazepam treatment is greatly reduced over time, with a therapeutic window of about 40 minutes post-exposure (McDonough et al., 1995; McDonough and Shih, 1993, 1997).

The effects of nerve agent exposure have been well studied in the central nervous system (CNS) of various animal models. Within 24 hours after soman or VX exposure, neuronal degeneration and death are observed in the piriform cortex, hippocampus, thalamus, and amygdala brain regions (McDonough et al., 1987; McLeod, 1985). Astrocytic activation and death are also observed within 24 hours of exposure (Zimmer et al., 1997; Zimmer et al., 1998). Cell death appears to be biphasic, with an initial necrotic phase responsible for the majority of the neuronal death followed by an apoptotic phase in which a subpopulation of neurons that survive the necrotic phase are cleared (Kan et al., 2006). More recently, molecular characterization of CNS tissue has indicated a regional and temporal neuroinflammatory response following nerve agent-induced seizure, presumably due to the resulting cellular death (Johnson et al., 2011; Johnson and Kan, 2010; Spradling et al., 2011a, b).

Although neuronal and astrocytic cell death is readily observable *in vivo* following acute exposure to soman or VX, nerve agent-induced cell death has been difficult to observe in cultured neurons, and there are no published studies investigating nerve agent-induced death in cultured astrocytes. Sawyer and colleagues showed that soman, sarin, cyclosarin, tabun, and VX inhibit acetylcholinesterase in mouse embryonic cortical neuron cultures, but did not report agent-induced cell death (Sawyer et al., 1991). In agreement, primary cultures of rat hippocampal or cortical neurons exposed to soman showed nearly complete inhibition of acetylcholinesterase without effect on cell viability (Deshpande et al., 1995). In contrast, Kanjilal and colleagues used differentiated NSC-34 cells, a cell line produced by fusing motor neuron-enriched embryonic spinal cord cells with mouse neuroblastoma cells, to demonstrate VX-induced cell death mediated by caspase-3 activation. The caspase-3 activation was attenuated by addition of the oxime pralidoxime (2-PAM) or the N-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (Kanjilal et al., 2014). Tenn and Wang also report VX-

induced cell death mediated by caspase-3 activation, but they used primary cultures of rat cortical neurons (Tenn and Wang, 2007). In a following study, VX-induced cell death was shown to be attenuated by an NMDA receptor antagonist, but not by α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor or metabotropic glutamate receptor antagonists (Wang et al., 2008). In addition, physostigmine (reversible acetylcholinesterase inhibitor) or atropine (muscarinic receptor antagonist) treatment did not attenuate VX-induced neuronal death, indicating a non-cholinergic mechanism for agent-induced cell death. To further investigate potential non-cholinergic effects of nerve agents, we examined the gene expression changes induced by nerve agent exposure in primary cultures of human neurons and astrocytes.

Materials and Methods

Human Neuron and Astrocyte Cell Culture and Agent Exposure

Human neurons were purchased from ScienCell Research Laboratories (Carlsbad, CA) and grown according to manufacturer's instructions. Human astrocytes were purchased from Lonza, Inc. (Allendale, NJ) and grown according to manufacturer's instructions in T25 flasks. Soman and VX were obtained from the United States Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD) and diluted in saline at the United States Army Medical Research Institute of Chemical Defense (Aberdeen Proving Ground, MD). Cultured cells were exposed to 100 µM of agent for 1 hour at 37 deg C. Concentrations greater than 100 µM of agent were not tested to remain within possible physiological concentrations and for safety considerations. Neuron and astrocyte cultures were washed five times with media and phosphate buffered saline, respectively, to remove unbound agent. Media was added after the fifth wash, and 24 hours after exposure, the cells were harvested in RLT buffer (Qiagen, Inc. Valencia, CA) for RNA isolation. Neuronal and astrocytic cell death was not observed under these conditions, and extended exposure times (up to 6 hours) at 100 µM did not induce cell death observable by propidium iodide staining and flow cytometry (data not shown).

RNA Preparation for Microarray Analysis

Total RNA was isolated from the cultured astrocytes or neurons using the Qiagen RNeasy kit according to the manufacturer's instructions (Qiagen, Inc.). The quality and amount of RNA was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA) and a NanoDrop ND-1000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA), respectively. As previously described (Spradling et al., 2011b), isolated RNA was processed for hybridization to Human Genome U133 Plus 2.0 oligonucleotide microarrays (Affymetrix, Inc., Santa Clara, CA) using the BioArray Single-Round RNA Amplification and Biotin Labeling System (Enzo Life Sciences, Inc., Farmingdale, NY). Processing of the biotinylated cRNA from each sample was performed using the Affymetrix GeneChip Instrument System according to the manufacturer's protocol (Affymetrix, Inc.).

Microarray Data Analysis

Raw data (signal intensities) for each GeneChip were imported into Partek Genomics Suite v6.6 beta (Partek, Inc., St. Louis, MO) and normalized using robust multi-array averaging (RMA). Normalized data were analyzed by principal component analysis (PCA) to identify patterns and major sources of variability in the data. Analysis of variance (ANOVA) identified genes most significantly affected by nerve agent exposure (fold change ≥ 1.5 or ≤ -1.5, p-value ≤ 0.05). Probeset identification, p-value, and fold change were imported into Ingenuity Pathway Analysis (IPA; Qiagen, Inc., Redwood City) software to identify the canonical pathways, biological functions, and networks of genes significantly altered by agent exposure of cultured neuronal cells.

Canonical pathway analysis identified the pathways from the IPA library of canonical pathways that were most significant to the data set. Molecules from the data set that met the p-value cutoff of ≤ 0.05 and were associated with a canonical pathway in the Ingenuity Knowledge Base were considered for the analysis. The significance of the association between the data set and the canonical pathway was measured in 2 ways: 1) A ratio of the number of molecules from the data set that map to the pathway divided by the total number of molecules that map to the canonical pathway, and 2) Fisher's exact test was used to calculate the probability of an association between the genes in the dataset and the canonical pathway.

The IPA functional analysis identified the biological functions and/or diseases that were most significant to the data set. Molecules from the dataset that met the p-value cutoff of ≤ 0.05 and were associated with biological functions and/or diseases in the Ingenuity Knowledge Base were considered for the analysis. Right-tailed Fisher's exact test was used to calculate the probability of each biological function and/or disease assigned to that data set. The molecules associated with the identified biological functions and/or diseases were then overlaid onto a global molecular network developed from information contained in Ingenuity's Knowledge Base. Networks of molecules were then algorithmically generated based on their connectivity.

Results

Gene expression profiles in cultures of human neurons and astrocytes are altered by exposure to soman, but not VX.

Normalized microarray data from soman- or VX-exposed astrocyte and neuron cultures were analyzed by PCA to determine major sources of variability within the respective datasets (Figures 1 and 2). For the PCA plots, each point represents a gene expression profile from a sample in the dataset. Points nearer in distance on the plot have similar gene expression profiles, whereas points further in distance on the plot have differing gene expression profiles. The PCA plot for the cultured astrocytes indicated exposure date, but not agent exposure as a source of variability in the dataset (Figure 1). In agreement, very few transcriptional changes were observed in response to either soman or VX exposure. An ANOVA with p-value and fold-change limits of 0.05 and ±1.5, respectively, revealed expression changes in only 1 transcript for VX and 60 transcripts for soman compared to control samples.

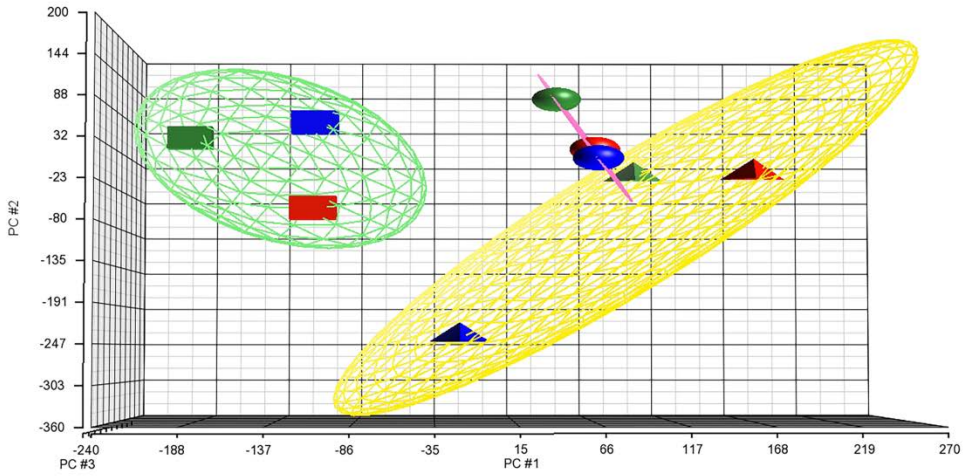

Figure 1: PCA of astrocyte gene expression profiles reveals exposure date but not agent exposure as the major source of variation. *(1.5 column)* Principal component analysis (PCA) groups samples based on their similarities and differences. Points on the PCA close in proximity share more similarities than points grouped farther away. The PCA describes 59.6% of the variability in the data set (PC #1 25.5%; PC#2 19%; PC#3 15.2%). Samples are colored to indicate agent (control, red; soman, green; VX, blue); shape and ellipsoid color indicate exposure date (exposure 1, sphere and pink; exposure 2, pyramid and yellow; exposure 3, cube and green, respectively).

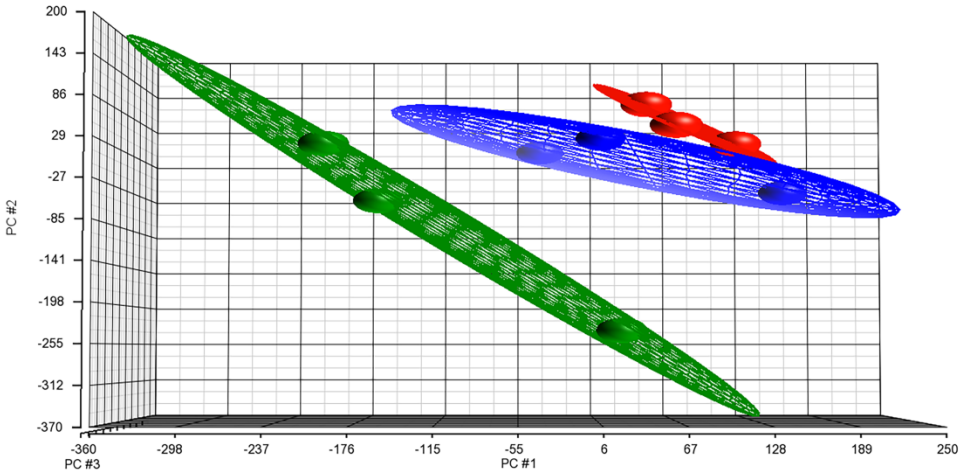

Figure 2: PCA of neuron gene expression profiles reveals agent exposure as a source of variation. *(1.5 column)* The PCA describes 54.6% of the variability in the data set (PC #1 25.5%; PC#2 17.4%; PC#3 11.7%). The VX (blue) and control (red) points on the PCA are in close proximity, indicating few differences in gene expression. However, soman samples (green) are grouped farther from both the VX and control samples, indicating differences in gene expression.

In contrast to the astrocyte, PCA of gene expression profiles for neurons indicated agent exposure as a source of variability in the data (Figure 2). The points on the PCA plot corresponding to the VX-exposed samples are in close proximity to the control samples, indicating few gene expression changes. In agreement, only two gene expression changes for VX-exposed neurons are revealed following ANOVA. However, the points on the PCA corresponding to the soman-exposed samples are clearly separated from both the VX-exposed and the unexposed samples, suggesting significant gene expression changes in response to this agent. ANOVA revealed 464 genes differentially expressed following soman exposure of neuronal cultures.

Identification of significant canonical pathways suggests changes in inflammatory response following soman exposure of cultured human neurons.

IPA software was used to identify the canonical pathways significantly altered by soman exposure of cultured human neurons. The canonical pathways in the Ingenuity Knowledge Base are accepted signaling or metabolic pathways established in the published literature. Forty canonical pathways were identified as significantly affected following soman exposure (Figure 3 and data not shown). Twelve of the identified canonical pathways include inflammatory components. Most common to these 12 pathways were increases in gene expression of tumor necrosis factor receptors 1A or 11B (*TNFRSF1A* and *TNFRSF11B*, respectively), interleukin 1 receptor type I (*IL1R1*), signal transducer and activator of transcription 3 (*STAT3*), interleukin 6 signal transducer (*IL6ST*) and/or chemokine C-C motif ligand 2 (*CCL2*; also known as macrophage chemotactic factor 1 or MCP-1). These data suggest that soman exposure may directly alter the gene expression of inflammatory response proteins in neurons.

Soman-induced neuronal gene expression changes associate with neurological disease and inflammatory response.

Using IPA, the functional analysis of the dataset for soman-exposed neurons identified biological functions relating to neurological disease and inflammatory response as significant. The ten most significant neurological disease functions associated with soman exposure in neurons are listed in Table 1. The most significant neurological disease was encephalopathy (p-value = 7.74E-13), which also had the highest number of associated transcripts in the data set (93 transcripts). Interestingly, the top seven neurological disease categories include the γ-aminobutyric acid (GABA) A receptor alpha 5 subunit (*GABRA5*) gene, which was increased 1.957-fold (p-value = 0.0173). For the inflammatory response within functional annotation analysis of the soman-exposed neurons (Table 2), the most significant function was categorized as inflammation (p-value = 3.81E-12; 54 transcripts), but the annotation with the highest number of associated transcripts was identified as immune response (p-value = 1.64E-09; 95 transcripts). Of the top ten inflammatory response functions, the first nine include *TNFRSF1A*, which was increased 1.914-fold (p-value = 0.0173). The identified molecules in the dataset were used to generate networks of genes affected by soman exposure. The gene networks associated with encephalopathy (Figure 4) and immune response (Figure 5) demonstrate overlapping involvement of several transcripts, including *TNFRSF1A* and *IL1R1*.

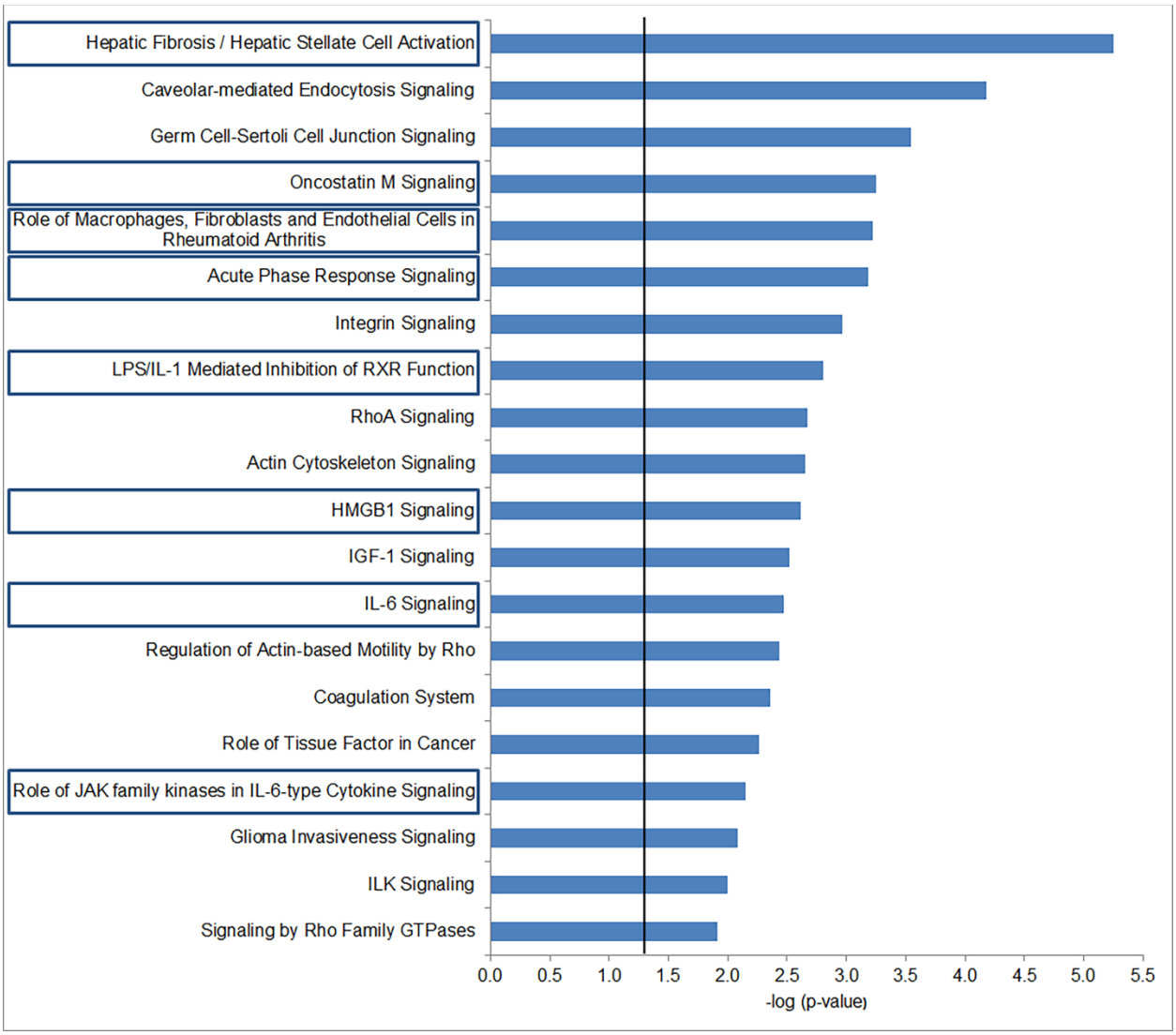

Figure 3: Canonical pathway analysis for soman-induced gene expression changes in cultured human neurons. *(2 column)* The top 20 significant canonical pathways are shown. The p-value cutoff (0.05) is indicated by the black line. Canonical pathways boxed in blue contain inflammatory molecules.

Table 1: Soman-induced neuronal gene expression changes in biological functions of neurological disease. *(1.5 column)*

Functions Annotation	p-Value	No. of Transcripts
Encephalopathy	7.74E-13	93
Neuromuscular Disease	4.29E-08	67
Movement Disorder	2.59E-07	66
Neurological Signs	1.21E-07	52
Dyskinesia	1.62E-07	51
Huntington's Disease	2.45E-07	49
Dementia	2.60E-04	33
Encephalomyelitis	1.28E-08	25
Experimental Autoimmune Encephalomyelitis	5.10E-08	24
Brain Cancer	1.90E-05	21

Table 2: Soman-induced neuronal gene expression changes in biological functions of inflammatory response. *(1.5 column)*

Functions Annotation	p-Value	No. of Transcripts
Immune Response	1.64E-09	95
Inflammation	3.81E-12	54
Inflammation of Organ	5.95E-10	40
Inflammatory Response	2.88E-04	40
Cell Movement of Phagocytes	2.25E-07	39
Cell Movement of Neutrophils	1.67E-07	27
Quantity of Phagocytes	8.43E-05	27
Migration of Phagocytes	4.04E-06	23
Inflammation of Lung	4.45E-07	20
Response of Phagocytes	1.05E-05	18

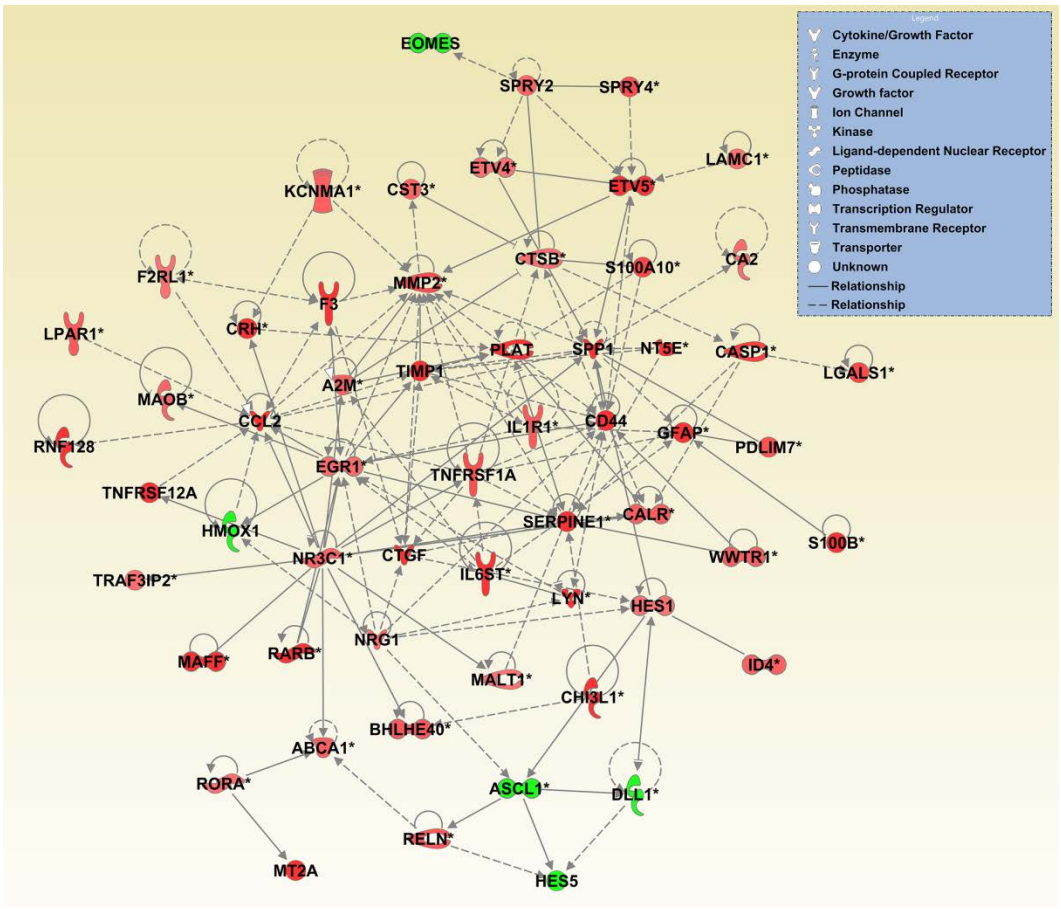

Figure 4: Soman-induced changes of neuronal gene expression involved in encephalopathy. *(1.5 column)* The gene network was generated in IPA by overlaying the molecules in the data set associated with the identified disease state (encephalopathy) onto a global molecular network developed from information contained in Ingenuity's Knowledge Base. Networks of molecules were then algorithmically generated based on their connectivity. Transcripts are represented by variously shaped nodes indicating functional class. Biological relationships between transcripts are indicated by lines. Red and green indicate increase or decrease in gene expression, respectively.

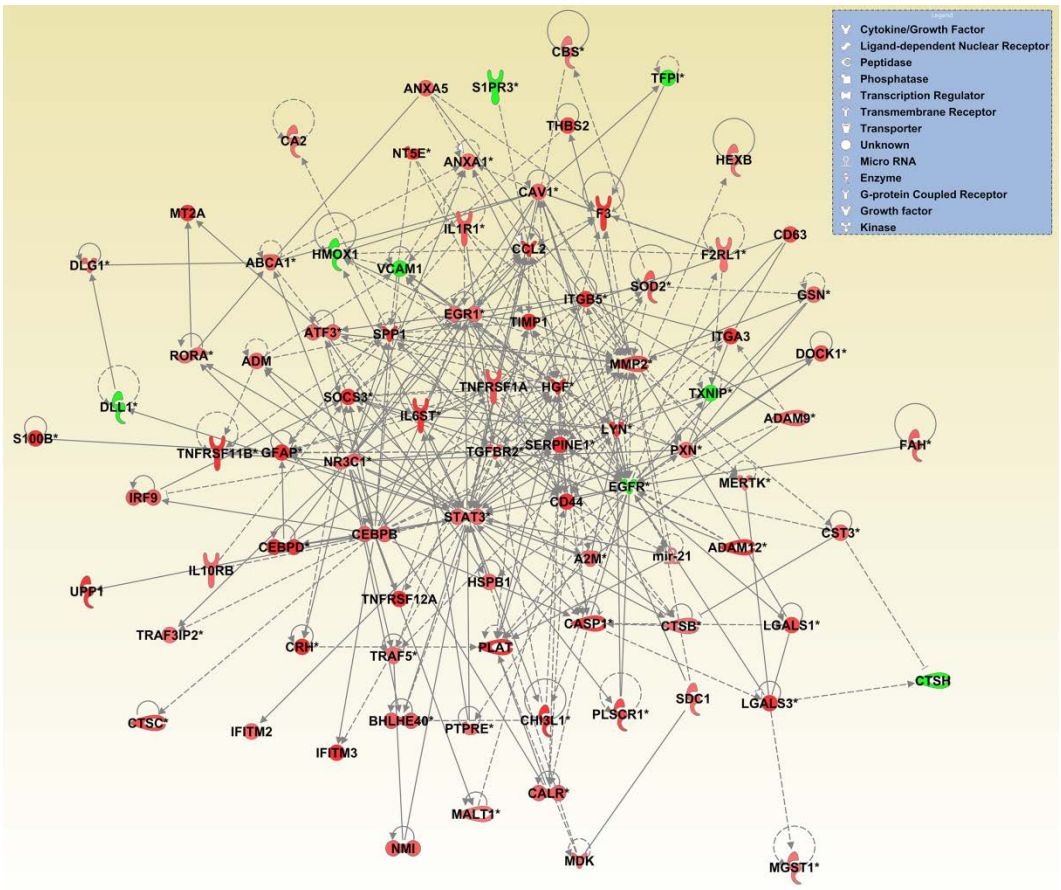

Figure 5: Neuronal expression changes of transcripts involved in immune response following soman exposure. *(1.5 column)* Transcripts are represented by variously shaped nodes indicating functional class. Biological relationships between transcripts are indicated by lines. Red and green indicate increase or decrease in gene expression, respectively.

Discussion

Demonstrating direct toxic effects of nerve agents on cultured cells has been difficult. Reports of VX-induced cell death in neuronal cultures indicated a role for programmed cell death pathways initiated by mechanisms other than acetylcholinesterase inhibition (Tenn and Wang, 2007; Wang et al., 2008). Although apoptotic death does occur after acute exposure to nerve agent *in vivo*, most of the brain injury is thought to occur via necrotic cell death (Kan et al., 2006). Cell death following acute exposure to nerve agent has largely been attributed to seizure activity (McDonough and Shih, 1997; Shih et al., 2003). However, the direct molecular effects of nerve agent exposure in the absence of seizure activity are difficult to demonstrate in animal models. To further investigate the molecular effects of nerve agent on cultured neuronal cells, a transcriptomic approach was initiated to identify networks of genes, canonical pathways and biological functions altered in the absence of nerve agent-induced seizure.

As mentioned previously, studies have been conducted to determine toxicity of nerve agents on cultured neurons. However, no studies have previously been published to investigate effects on astrocytes. Astrocytes are specialized glial cells in the brain that also express acetylcholinesterase (Bond et al., 2006). They are the most numerous cell type in the CNS, with an average of 10 astrocytes for every neuron in most brain regions (Magistretti and Pellerin, 1999; Tsacopoulos and Magistretti, 1996). Astrocytes perform a variety of functions that are essential for neuronal survival, including providing nutrients (e.g., glucose conversion to lactate and secreted for neuronal uptake), regulating extracellular ion (e.g., potassium) and excitatory neurotransmitter concentrations, producing pro- and anti-inflammatory molecules, producing growth factors, scavenging free radicals, and repairing the CNS after injury (Myer et al., 2006; Sofroniew, 2005). In response to oxidative stress, astrocytes can express a secreted form of acetylcholinesterase to control acetylcholine levels in the synaptic cleft (Bond et al., 2006). Since astrocytic death is also observed *in vivo* following an acute exposure to nerve agent and astrocytes express acetylcholinesterase, we investigated the effects of nerve agent exposure on cultures of human astrocytes.

PCA of astrocyte transcriptomic data indicated exposure date as a source of variation instead of agent exposure (Figure 1). The pattern of PCA points indicated few, if any, gene expression changes following nerve agent exposure of astrocyte cultures (Figure 1), and nerve agent-induced astrocytic death was not observed (data not shown). In agreement with this analysis, exposure to VX resulted in too few genes for mapping to canonical pathways. In addition, exposure of cultured human astrocytes to sarin or VR also resulted in very few gene expression changes (data not shown). Astrocytes exposed to soman displayed only 60 gene expression changes which were mapped to canonical pathways (data not shown). However, the canonical pathways identified as statistically relevant consisted of various signaling pathways. The neuronal reelin signaling pathway was the only pathway identified that was specific to the CNS. These data suggest that nerve agent exposure does not directly cause astrocytic death and directly induces few, if any, gene expression changes in astrocytes.

In agreement with previously published reports (Deshpande et al., 1995; Sawyer et al., 1991), soman did not induce cell death in the human neuron cultures (data not shown). However, in contrast to previous reports (Kanjilal et al., 2014; Tenn and Wang, 2007; Wang et al., 2008), VX did not induce cell death in the human neuron cultures either (data not shown). This discrepancy may be due to differences in the source of neurons (mouse and rat versus human) or in diluents for the VX (ethanol versus saline). In addition to a lack of neuronal death, the PCA for the neuronal cultures indicated few gene expression changes for VX exposure (Figure 2). This result differs from that recently published by Gao and colleagues (Gao et al., 2013). They demonstrated 513 and 1,451gene expression changes in human hN2 neural cells at 24 hours following exposure to 0.1 and 10 µM VX, respectively. The human hN2 cells in that study were differentiated from the human embryonic stem cell WA09 line. The differences in genomic response observed in this study may be attributed to differences in source of neurons (primary versus stem-cell derived).

In contrast to the observed VX data, the soman samples grouped distinctively from the VX and unexposed neuronal samples, indicating significant differences in gene expression. In

agreement, over 400 gene expression changes were observed following soman exposure. Although the top canonical pathways were not specific for neuronal function, many pathways had a common pattern—a clear inflammatory component (Figure 3). In support of this, functional analysis of the data indicated a significant role of the neuronal gene expression changes in inflammatory response (Table 2).

Neuroinflammation following many types of brain injury, including nerve agent-induced seizure, has been well documented (Johnson et al., 2011; Johnson and Kan, 2010; Toulmond et al., 1996; Vezzani and Granata, 2005; Wang and Shuaib, 2002). Following a seizurogenic dose of soman, chemokine (C-X-C motif) ligand 1 (CXCL1), macrophage inflammatory protein-1α (MIP-1α), interleukin (IL)-6, and MCP-1 are expressed in neurons *in vivo* ((Johnson et al., 2011) and EA Johnson, personal communication). Other inflammatory molecules including IL-1α, IL-1β, and tumor necrosis factor-α (TNF-α) are also upregulated in the CNS following soman-induced seizure (Johnson and Kan, 2010). In agreement, Dillman and colleagues (Dillman et al., 2009) also report increases in IL-6 and TNF-α gene expression in the hippocampus following soman exposure (summarized in Table 3). Increases in these inflammatory molecules are not unique to soman exposure. Previous studies have also indicated increases in *IL-6*, *TNF-α*, and *IL-1β* following sarin exposure (Spradling et al., 2011b). Interestingly, cultured human neurons exposed to soman display increased gene expression of *TNFRSF1A* and *IL1R1* (Figure 5), the major receptors for TNF-α and IL-1α or IL-1β, respectively. In addition, soman-exposed cultured neurons have increased expression of the MCP-1 gene (*CCL2* in Figure 5). Further analysis of the *in vivo* microarray data from Spradling and colleagues also reveals increases in *TNFRSF1A* and *11B* and *STAT3* (Spradling et al., 2011a, b), similar to our *in vitro* findings. The increase in *TNFRSF1A* and *IL1R1* expression may have a priming effect, making neurons more excitable.

Table 3: Comparison of *in vitro* and *in vivo* expression of inflammatory molecules. *(2 column)*

***In Vitro* Neuron Microarray**	***In Vivo* Brain Region Microarray***	***In Vivo* Brain Region Immunoassay†**
TNF Receptor 1A/11B	TNF Receptor 1A/11B; TNF alpha	TNF alpha
IL-1 Receptor Type 1	IL-1 beta	IL-1 alpha; IL-1 beta
IL-6 Signal Transducer	IL-6	IL-6
STAT3	STAT3; IL-6	IL-6
MCP-1	MIP-1 alpha	MCP-1; CXCL1; MIP-1 alpha

*From Spradling et al., 2011a, b

†From Johnson and Kan 2010, Johnson et al., 2011, and EA Johnson, personal communication

IL-1β and TNF-α, ligands for TNFRSF1A and IL1R, are proconvulsants (Stellwagen et al., 2005; Vezzani et al., 1999; Vezzani and Granata, 2005) that are expressed in the piriform

cortex, thalamus and hippocampus following soman-induced seizures (Johnson and Kan, 2010). IL-1β promotes seizures by activation of IL-1 receptors, inducing Src kinase-mediated tyrosine phosphorylation of the NR2B subunit of the NMDA receptor and triggering NMDA-mediated calcium influx (Vezzani and Baram, 2007; Viviani et al., 2003). In addition, IL-1β can block γ-aminobutyric acid (GABA) type A receptor inhibitory neurotransmission (Wang et al., 2000). Moreover, the intracerebral injection of IL-1 receptor antagonist has powerful anticonvulsant effects (Vezzani et al., 2002). TNF-α contributes to seizures by causing exocytosis of glutamate AMPA receptors and endocytosis of GABA A receptors (Stellwagen et al., 2005).

In addition to inflammatory response, neurological disease was also identified as significant in the IPA functional analysis. The most statistically relevant neurological disease was encephalopathy (Figure 4). Like inflammatory response, the gene network generated from the neuronal data set for encephalopathy also included *TNFRSF1A* and *IL1R1*. In addition, the top seven functional annotations for the identified neurological diseases all have *GABRA5* as a member of the gene set. GABRA5 is one of six α subunit types that can form the GABA A ion channel. The benzodiazepine binding site on the GABA A receptor is located between the α and γ subunits. Diazepam, the benzodiazepine currently fielded in the United States for military treatment of nerve agent exposure, binds non-selectively to the GABA A receptor α subunit to open the ion channel, causing inhibitory effects (Atack, 2005). The increase in the expression of the *GABRA5* gene following soman exposure of cultured human neurons suggests that components of the GABA pathway may be directly altered by nerve agent exposure, and an in-depth analysis of *in vivo* transcriptomic data focusing on the GABA pathway may reveal new targets within that pathway for improved medical countermeasures.

In conclusion, these data indicate molecular alterations in neuronal cells induced by soman exposure in the absence of cell death and presumably of seizure activity. Comparison of *in vivo* microarray and protein data with the *in vitro* neuron microarray data (Table 3) suggests that the molecular changes observed in the primary human neuronal cultures may relate to *in vivo* molecular changes induced by soman. In addition, these data suggest that the neuroinflammatory response following soman-induced seizure activity may be increased by direct, non-cholinergic molecular alterations in gene expression induced by soman. Changes in neuronal gene expression of inflammatory molecules support the development of anti-inflammatory approaches to address neuroinflammation observed following soman exposure. In addition, the observed increase in *GABRA5* following nerve agent exposure of cultured neurons provides the rationale for development of novel therapeutics to target specific proteins in the GABA receptor complex.

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