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Magnetic resonance imaging analysis of long-term neuropathology after exposure to the nerve agent soman: correlation with histopathology and neurological dysfunction

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Abstract

Nerve agents (NAs) produce acute and long-term brain injury and dysfunction, as evident from the Japan and Syria incidents. Magnetic resonance imaging (MRI) is a versatile technique to examine such chronic anatomical, functional, and neuronal damage in the brain. The objective of this study was to investigate long-term structural and neuronal lesion abnormalities in rats exposed to acute soman intoxication. T2-weighted MRI images of 10 control and 17 soman-exposed rats were acquired using a Siemens MRI system at 90 days after soman exposure. Quantification of brain tissue volumes and T2 signal intensity was conducted using the Inveon Research Workplace and the extent of damage was correlated with histopathology and cognitive function. Soman-exposed rats showed drastic hippocampal atrophy with neuronal loss and reduced hippocampal volume (HV), indicating severe damage, but had similar T2 relaxation times to the control group, suggesting limited scarring and fluid density changes despite the volume decrease. Conversely, soman-exposed rats displayed significant increases in lateral ventricle (LV) volumes and T2 times, signifying strong cerebrospinal fluid expansion in compensation for tissue atrophy. The total brain volume (TBV), thalamic volume, and thalamic T2 time were similar in both groups, however, suggesting that some brain regions remained more intact long-term after soman intoxication. The MRI neuronal lesions were positively correlated with the histological markers of neurodegeneration and neuroinflammation 90 days after soman exposure. The predominant MRI hippocampal atrophy (25%) was highly consistent with massive reduction (35%) of neuronal nuclear antigen (NeuN⁺) principal neurons and parvalbumin (PV⁺) inhibitory interneurons within this brain region. The HV was significantly correlated to both inflammatory markers of GFAP⁺

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Author contributions

D.S.R. participated in research design. S.D.R., X.W., R.K., V.S., and D.S.R. conducted experiments. S.D.R., X.W., V.S., and D.S.R. performed data analysis. S.D.R. wrote the first draft. S.D.R., X.W., and D.S.R. edited or contributed to the writing of the manuscript.

Competing interests:

The authors declare no competing interests.

astrogliosis and IBA1⁺ microgliosis. The reduced HV was also directly correlated with significant memory deficits in the soman-exposed cohort, confirming a possible neurobiological basis for neurological dysfunction. Together, these findings provide powerful insight on long-term region-specific neurodegenerative patterns after soman exposure and demonstrate the feasibility of *in vivo* neuroimaging to monitor neuropathology, predict the risk of neurological deficits, and evaluate response to medical countermeasures for NAs.

Graphical abstract:

Our results demonstrate chronic MRI lesions and volumetric abnormalities in the hippocampus and other key areas of the brain in the soman model of nerve agent intoxication. Moreover, these long-lasting changes are positively correlated with the histological markers of neurodegeneration and neuroinflammation in the hippocampus, and can predict the special learning and memory dysfunction. Together, these findings support the application of MRI as a sensitive and versatile *in vivo* neuroimaging tool to gain early insight into the anatomic extent and spatial progression in morphological defects in the brain after nerve agent exposure.

Keywords

epilepsy; hippocampus; MRI; memory; neuroimaging; nerve agent; organophosphate; soman

Introduction

Chemical warfare nerve agents (CWNAs) are weapons of mass destruction for attacking military and civilian populations. Nerve agents (NAs; e.g., soman and sarin) and organophosphates (OPs, e.g., diisopropyl-fluorophosphate (DFP)) are highly neurotoxic agents.¹⁻³ These chemical agents are considered the most lethal among chemical weapons and produce acute and long-term neurotoxicity.⁴⁻⁷ CWNAs are neurotoxic primarily owing to their irreversible inhibition of acetylcholinesterase, leading to an excessive accumulation of acetylcholine in the brain. Acute exposure to CWNAs causes a set of predictable acute toxic signs: hypersecretion, fasciculations, tremors, convulsions, respiratory distress, and death.^{8, 9} Convulsive seizures and status epilepticus (SE) may result in death or long-term neuronal damage.¹⁰⁻¹² Controlling persistent seizures at an early stage is critical for survival and preventing long-term neurological dysfunction after chemical intoxication.^{13, 14} As per animal studies, effective antidotes must be given within 30 min for protection against seizures and neurological damage.¹⁵⁻¹⁷ This timeline is often not practical in many emergencies. Therefore, CWNA-induced seizures and neurotoxicity can lead to long-term brain injury and devastating neuropsychiatric dysfunction in survivors of chemical attacks^{18, 19} and animal models.¹²

Survivors of a CWNA attack suffer from a number of devastating, long-term neurological problems.⁷ According to the 2019 report by the U.S. National Toxicological Program,²⁰ acute sarin exposure is known to be a neurological hazard to humans in the initial (1–7 days), intermediate (8 days to 1 year), and extended (1 year) times after exposure. This risk assessment is based on multiple physiological effects, including suppression of AChE, visual and ocular effects, and morphological and histological changes in the brain.²⁰ Five years

after the attacks with the nerve agent sarin in Matsumoto and Tokyo, exposed individuals reported persistent increases in neurobehavioral disorders, trauma, and insomnia.^{19, 21-23} Novel medical countermeasures are needed to prevent these short- and long-term effects of CWNAs.²⁴⁻²⁶

Magnetic resonance imaging (MRI) is an increasingly popular *in vivo* imaging technique for investigating anatomic, functional, and neuronal lesions in the brain. MRI is a significant asset for cases such as CWNA exposure, where a specific diagnosis is crucial for therapeutic intervention in exposed persons.²⁷ Typical MRI features of CWNA exposure include specific brain region atrophy, disrupted internal structure, and increased T2-weighted signal. Quantitative volumetry can quantify volume loss (atrophy), representing neuronal cell loss using T2 relaxometry that measures T2 relaxation time, an intrinsic tissue property.²⁷ Moreover, given its noninvasive nature, the same patient can be imaged longitudinally to observe a time course of changes in the brain and expected behavioral symptoms. T2-weighted images, and their associated T2 relaxation times, are frequently used to identify anatomical changes, such as tissue loss or presence of lesions in landmark regions, such as the hippocampus.^{28, 29} Diffusion tensor imaging has been used to generate apparent diffusion coefficient (ADC) maps to identify symptoms, such as restricted diffusion or edema.^{30, 31} CWNA exposure is known to cause rapid damage to the hippocampus, which is involved in major pathways associated with memory function and epilepsy³²⁻³⁴, even compared with other brain structures. The thalamus is another susceptible brain structure, known to be affected with cell loss within hours of chemical exposure.³⁵ However, there have been limited imaging studies of long-term brain abnormalities resulting from CWNA intoxication. Moreover, acute or transient changes in T2 relaxation time tend to fade within a week.²⁹ Therefore, we expect to observe clear pathological changes (e.g., neuronal damage and atrophy) in the prime regions of interest (ROIs) for chronic neurological effects, particularly in the hippocampus, but no change in T2 relaxation times observed during the acute stage.

In this study, we sought to utilize MRI to investigate long-term structural and neuronal lesion abnormalities in rats with acute soman intoxication. Cognitive dysfunction is a common feature in many victims of nerve agent exposure, but the neurobiological basis of these long-lasting symptoms are unclear. Therefore, we correlated the long-lasting MRI lesions with histological neurodegeneration and neuroinflammation in the hippocampus, and we directly related these changes to hippocampus-mediated spatial memory dysfunctions in animals 3 months after soman exposure.

Materials and methods

Animals

Adult male Sprague–Dawley rats (3 months old; 250–300 g; Taconic Farms, Rockville, MD) were used in the study. They were housed in an environmentally controlled animal facility with a 12-h light:dark cycle. Animals were utilized for experiments after 1 week of acclimatization to the vivarium. All procedures were performed in compliance with the guidelines of the NIH Guide for the Care and Use of Laboratory Animals under a protocol approved by the university's Institutional Animal Care and Use Committee.

The soman exposure model

The overall experimental protocol for soman exposure and the timeline for various tests is illustrated in Figure 1. The soman exposure studies were conducted at the MRIGlobal facility (Kansas City, MO). Rats were acclimatized to the lab conditions for at least 5 days before experimental studies. Animals were exposed to soman (154 µg/kg, $1.4 \times \text{LD}_{50}$) by a single subcutaneous injection, as per the previously published protocol.^{10, 16, 36} HI-6 (asoxime chloride; 125 mg/kg, intraperitoneally (i.p.)), an oxime reactivator of cholinesterase, was administered 30-min prior to soman to increase the survival rate. HI-6 therapy can reduce soman-induced serious cardiovascular complications, respiratory compromise, and death. Within 1 min of soman exposure, rats were treated with atropine methylnitrate (2 mg/kg, intramuscularly (i.m.)) to minimize peripheral cholinergic toxic effects. The LD_{50} values of soman and protective drug treatment regimen are consistent with the previously published reports in rat models by Apland and colleagues.^{10, 16, 36} The anticonvulsant midazolam (2 mg/kg, i.m.) was administered 40 min after soman exposure for controlling acute seizure activity.

Soman (pinacolyl methylphosphonofluoridate; MRIGlobal, Kansas City, MO) was diluted in cold saline and administered to rats via a single subcutaneous injection. Ketoprofen injection (100 mg/mL, cat. no. 07-803-7389) and midazolam injection solution (10 mg/mL, cat. no. 07-890-6698) were purchased from Paterson Veterinary (Houston, TX). Midazolam was diluted in sterile saline prior to the injection. Atropine methyl nitrate powder (cat. no. ASB-00111075-250) was purchased from ChromaDex (Irvine, CA). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO). HI-6 (1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxapropane dichloride) was obtained from Kalexsyn, Inc (cat. no. KLXN-1618, Kalexsyn, Kalamazoo, MI). The test drug solution was administered at a volume not to exceed 0.1 mL per 100 mg body weight of the animal.

Animals were monitored by the video encephalography system (Grass-Astromed, Warwick, RI) for 24 h to check encephalographic (EEG) and behavioral seizure activity and relevant clinical behaviors, such as respiration pattern and motor activity state.³⁷ The severity of behavioral seizures were rated according to the Racine scale:³⁸ stage 0, normal behavioral activity; stage 1, chewing and facial twitches; stage 2, head nodding or shaking; stage 3, unilateral forelimb clonus without rearing, Straub tail, and extended body posture; stage 4, bilateral forelimb clonus plus rearing; and stage 5, wild jumping and tonic-clonic activity. After 2 weeks of recovery, rats were transported to a Texas A&M animal facility for long-term monitoring and MRI scanning. The MRI scans were taken to evaluate the long-term impact of soman exposure at 3 months postexposure based on our pilot studies in the DFP intoxication model.²⁷

MRI acquisition

MRI scans were performed in 10 healthy controls and 17 soman-exposed surviving rats around 90 days after exposure to soman. Rats were anesthetized by an i.p. injection of a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg). MR images were generated on a 3-Tesla Siemens MAGNETOM® B17 Verio scanner with a 15-channel coil modified with

inserted polyvinyl chloride tubing to contain rats with minimal air gaps. Analyzed MRI sequences included (1) T2-weighted coronal dual-echo fast spin-echo with echo time (TE) 27/123 ms, repetition time (TR) 3610 ms, slice thickness 1.5 mm, field-of-view (FOV) 52×64 , and matrix 208×256 ; and (2) T2-weighted sagittal spin-echo with TE 37 ms, TR 2600 ms, slice thickness 1.5 mm, FOV 52×64 , and matrix 166×256 . Anatomical imaging with MRI is most commonly achieved by unique relaxation times (T1 and T2), which characterize tissue damage and pathologies.³⁹ T1 or T2 weighting is accomplished by adjusting timing parameters in MRI pulse sequence generation. T1 weighting is typically used in neuroimaging to assess anatomical detail, whereas T2 weighting is primarily used to evaluate pathological lesions or neuronal atrophy²⁷. Hence, we utilized a T2-weighted signal for detecting structural changes in the brain, especially to identify region-specific neuronal damage patterns. In the brain, grey and white matter have distinct T1 and T2 signals from each other and from the cerebrospinal fluid (CSF), which produces good contrast for anatomical imaging. T2 relaxation can reveal subtle pathology, even in the absence of tissue atrophy, and appears to be the most consistent imaging measurement in animals exposed to OP agents.²⁷ Owing to the cost and time constraints of neuroimaging acquisition and analysis of data in rat models, we conducted MRI imaging at one time point around 90 days after exposure to soman. These animals were not exposed to progressive MRI scans or other procedures involving multiple ketamine injections. Behavioral tests, such as the water maze cognitive paradigm, were conducted as described below.

MRI analysis

Quantification of brain tissue volumes and T2 signal intensity (SI) was conducted on a Siemens Inveon Research Workplace by marking ROIs on a per-voxel basis.⁴⁰ Acquired MRI images were preprocessed on Siemens Syngo workstations before being uploaded onto the specialized Inveon software. Regional BVs, as well as T2 signal intensities, were measured on the multiplanar images of the brain, using the ROI method of analysis. The programmable algorithms, which are an integral part of the Inveon software provided by the manufacturer (Siemens Preclinical Solutions, Knoxville, TN), automatically calculated T2 signal intensity. ROIs included the hippocampus, thalamus, and LV regions in four coronal slices located approximately 0.7, 2.2, 3.7, and 5.2 mm posterior to the bregma. Additionally, separate ROIs representing the entire brain (sans olfactory bulb and spinal cord) were drawn in all 10 sagittal slices to determine whole brain volumes (WBVs). ROI location determination was guided by Paxinos and Watson's *Rat Brain Atlas*.⁴¹ T2 relaxation times in dual-echo coronal slice ROIs were calculated using the expression $(TE_2 - TE_1) / \ln(SI_1/SI_2)$, where TE_1 (27 ms) and TE_2 (123 ms) denote the respective echo time and SI_1 and SI_2 are signal intensity at echo times TE_1 and TE_2 .⁴² Hippocampal and other brain region volumes were calculated using the built-in analysis tool in the Inveon software. HVs were also normalized to the TBV and expressed as a percentage for comparison across time and groups. Generalized linear models are often used to control variance in the WBV owing to mislabeling or misplacements in sub-brain ROI borders. The overall outcomes are similar between the relative versus linear quantification approaches, indicating the reliability of the brain volumetry technique.

Brain histology

Rats were perfused to fix the brain for histological analysis around 3 months after exposure to soman. Rats were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) injection, then transcardially perfused with normal saline followed by 4% paraformaldehyde solution in sodium phosphate buffer (pH 7.4). The brain was excised and postfixed as described previously.⁴³ To analyze the overall neuronal loss and neurodegeneration in brain slices, control ($n = 8$) and soman-exposed ($n = 8$) groups were euthanized after 90 days postexposure and brain tissues were processed for immunostaining as described previously.⁴⁴ Serial coronal (30 μ m) sections were cut through the forebrain containing the hippocampus, piriform cortex (PC), and amygdala relative to the bregma -0.24 mm to -7.44 mm.⁴¹ The sections were collected serially in a 24-well plate with cold PBS. Every 20th section (600 μ m intervals) through the entire hippocampus was then selected from each rat (total 10 sections per animal) and processed for immunohistochemistry (IHC).^{37,44} All sections were stored free-floating in section storage solution at 22 °C before further processing. The extent of neuronal damage and neuronal cell loss were assessed in the brain sections using three markers: (1) neuronal injury (Cresyl violet (Nissl)); (2) principal neuronal loss (neuronal nuclei (NeuN)); and (3) interneuron loss (parvalbumin (PV)) in hippocampal subfields. The extent of neuroinflammation efficacy was assessed by IHC using two specific markers: (1) astrocyte activation/injury (glial fibrillary acidic protein (GFAP)); and (2) microglia activation/injury (ionized calcium-binding adaptor molecule-1 (IBA1)) in hippocampal subfields.

Stereology quantification of neuronal cell loss

Design-based stereology was used to quantify the total number of neurons and the percentage of neuronal cell loss in various brain regions, as previously described.⁴³ The absolute cell counts in the hippocampus were calculated using the optical fractionator component of the Visiopharm[®] software. The stereology system consisted of the newCAST[®] software (version VIS 4.0; Visiopharm, Hørsholm, Denmark) and the Olympus BX53 microscope (Olympus, Tokyo, Japan) mounted with a color camera (DP73, Model: DP73-1-51; Olympus) and a CCD camera (ORCA-R2; Hamamatsu, Hamamatsu City, Japan). A detailed protocol for stereological quantification and analysis of neuronal cell loss, including the number of brain sections and the number of fields per brain section used for calculating the aggregate cell counts, was described previously by our lab.⁴³ Briefly, the extent of neurodegeneration was quantitated in different regions, such as the cornu ammonis (CA1, CA2, and CA3), dentate gyrus (DG), and dentate hilus (DH) of the hippocampus. Quantification of cell numbers was performed on one randomly selected side of the brain slices because the overall neurodegeneration was symmetrical in the brain.^{37,44}

The absolute numbers of immunopositive neurons (NeuN and PV) in different subfields of the hippocampus in multiple groups were counted by unbiased fashion. Subjective bias was minimized by blinded analysis of the histology sections and blinded compilation of the stereology data by the counters. The number of surviving neurons were quantified at 5% of the total region area for NeuN⁺ and PV⁺ cells at 60 $\times$ magnification in the CA1, CA3, and DG subfields. The total area was increased to 10% for NeuN⁺ and PV⁺ counts in the CA2

and DH subfields. The total area selection was based on the relative density cells in different brain regions to ensure optimal sampling for stereological analysis of cell counts.

Area fractionation densitometry quantification of neuroinflammation

For quantification of neuroinflammation, the extent of activation of GFAP⁺ astrocytes and IBA1⁺ microglial responses was assessed by area fractionation densitometry using ImageJ (the National Institutes of Health, Bethesda, MD).^{37,44} For each subfield, one image for each slice (at least five images for each rat) was taken in the same anatomic position at the coordinates relative to the bregma (e.g., -3.00 mm at anteroposterior axis), under the 20× objective lens. For measurements of area fraction of GFAP⁺ or IBA1(1) cells in ImageJ, each image was converted to 16-bit grayscale. A threshold value was selected to keep all the GFAP⁺ or IBA1⁺ expression in brain sections, but not the background area.³⁷ The area occupied by the cell structures was then evaluated by selecting the “Analyze Particles” component in ImageJ using identical particle parameters. This procedure estimated the area fraction of particles to the total area of the cells examined.

Spatial learning and memory test

The hippocampal-dependent spatial learning and memory function in soman-exposed rats was assessed by the Morris water maze test as described previously.^{45, 46} In this study, we selected the water maze test because it is one of the most robust tests for hippocampus-dependent spatial learning and memory retrieval. As outlined in the overall experimental paradigm (Fig. 1A), rats were subjected to Morris water maze training and memory paradigm around 90 days after exposure to soman. Rats were trained on a spatial memory task in the Morris water maze. The water maze consisted of a large circular tank painted black and filled with water at room temperature (20–22 °C) at the height of 35 centimeters. The water was rendered opaque by the addition of black, nontoxic paint, and extra-maze cues were positioned on the walls of the room around the tank. The pool was arbitrarily divided into four equal quadrants and a circular platform (a 15-cm diameter) was submerged 2 cm below the water surface in the middle of one quadrant (southwest quadrant of the maze). Black curtains with large white geometric designs present in the room provided the extra-maze spatial cues. Rat movement was acquired via a high-definition video camera mounted above the maze that was connected to a computer with a video tracking system. The rat was trained to find the platform using spatial cues for seven sessions over 7 days with four acquisition trials per session. Each rat was placed in the water, immediately facing the wall of the tank in one of the quadrants in a random manner, and then allowed a maximal time of 90 s to locate the platform with an intertrial interval of 120 seconds. If the subject reached the platform, it was allowed to stay there for 30 seconds. If the subject failed to find the platform within the ceiling time of 90 s, it was guided onto the platform and allowed to stay there for 30 seconds. For the spatial learning assessment, the location of the platform remained constant in one quadrant of the maze and the starting position for each trial was varied among four equally spaced positions around the perimeter of the maze. Mean times to reach the platform were used for the assessment of learning curves. Memory retrieval was assessed on day 8. Rats were subjected to a single 45-s probe or a memory retrieval test. This involved removal of the platform from the pool and release of rats from the quadrant that is diametrically opposite to the quadrant where the platform was positioned during

learning sessions. Retrieval of learned memory was assessed by comparing dwell time in the platform quadrant in control and soman-exposed groups. Memory retention was tested on day 15 as per the similar step protocols of memory retrieval.

Experimental outcomes

The study was designed to analyze the long-term effects of soman exposure on brain structural and volumetric abnormalities (Fig. 1). Rats were randomly assigned to the soman-exposed and control groups using the randomization sequence generation. The power and sample size were computed based on the proposed statistical tests, including one- and two-way repeated-measures analysis of variance (ANOVA) for neuropathological outcomes.⁴⁷⁻⁴⁹ A sample size of 10 was found to be adequate for MRI evaluation of brain regional defects in the soman exposure model. A larger sample size had been found to reduce the variability and increase the reliability of the imaging outcomes; therefore, a higher size was utilized for the soman-exposed group. A sample size of six or more was found to be sufficient for histopathological outcomes. For the water maze, we utilized 10 control and 17 soman-exposed rats. Atropine and midazolam were given as standard antidotes for improved survival of soman-exposed animals. A single-dose midazolam (2 mg/kg, i.m.) was given at 40 min after soman exposure, which is considered a critical period and simulation of a practical therapeutic window for first responders for emergency care in the case of chemical incidents.

Data analysis

Group data are expressed as the mean $\pm$ standard error of the mean (SEM). In all statistical tests, statistically significant differences were set at $P < 0.01$. Statistical comparisons of volumes and T2 relaxation outcomes were performed using one- or two-way repeated-measures ANOVA, as appropriate. Post-hoc analyses were carried out to identify specific differences using Tukey's honestly significant difference for multiple comparisons. Nonparametric outcomes, such as survival rate, were compared between groups using the Wilcoxon signed-rank test. Behavior seizure score and neuropathology score outcomes were analyzed with the nonparametric Kruskal–Wallis test followed by the Mann–Whitney U -test. The data from the Morris water maze test were analyzed by a two-way ANOVA followed by Dunnett's post-hoc t -test for two-group comparisons. A correlation analysis was performed to determine the relationship between MRI changes, such as the lesion volume and T2 signal intensity, and the extent of the histological neurodegeneration or the association between MRI lesions and memory dysfunction. For correlation analysis, Pearson's correlation coefficient r was calculated along with the P -value, testing the null hypothesis that there is no correlation between the two quantities. Pearson's correlation coefficient was calculated between the total HV (on days 5 and 15) and the performance in spatial memory tasks. All statistical tests, including multiple comparisons, were performed using SAS[®] software (SAS Institute Inc., Cary, NC) and OriginPro[®] 2020 (OriginLab Corporation, Northampton, MA).

Results

Soman-induced acute seizure characteristics

The animals were exposed to soman as per the published protocol, as illustrated in Figure 1A. Exposure of rats to soman triggered a very rapid cholinergic crisis, which was characterized by chewing and facial twitches (stage 1 on the Racine scale), then massive salivation, body twitches, and behavioral signs of chewing and head nodding (stage 2). These behavioral events quickly progressed into severe clonic seizures (stage 3), and finally generalized tonic-clonic seizure activity with rearing and falling (stage 5) at around 5–8 min after soman exposure. The acute seizure events progressed into persistent SE. Midazolam (2 mg/kg, im) was administered at 40 min postexposure to control seizures and SE. The 24-h seizure activity plot was illustrated in Figure 1B. These time-course results show that midazolam was partly effective at suppressing behavioral seizures when given 40 min after DFP exposure. A typical EEG trace was illustrated for soman-induced seizure activity in one rat with an implanted electrode (Fig. 1C). Long-term follow-up data were collected from all surviving animals ($n = 17$) from the soman-exposed group.

Soman-induced long-term alteration in the BV

To evaluate broad-scale alterations to a long-term BV caused by soman exposure, sagittal MR images were used for this measure since this is the shortest axis of the rat brain, and thus required fewer images to capture the entire brain. Representative sagittal slices for control and soman-exposed animals are given in Figure 2A. Overall, the sagittal morphometric images show a normal global BV in animals exposed to soman. Volumetric analysis shows of the TBV derived from the sagittal slices were not significantly different between control and soman-exposed groups (Fig. 2B). This view was least conducive to clearly observe tissue differences in the ROIs between groups. Therefore, a subsequent region-specific analysis was extracted from coronal slices.

Soman-induced long-term morphological alterations in the hippocampus

To determine long-lasting anatomical changes in the hippocampus following soman intoxication, coronal images were evaluated for volume and T2 relaxation times at 90 days postexposure. Representative coronal slices (from posterior to anterior) for control and soman-exposed animals are given in Figure 3. Hippocampal measurements were taken using slices 8–10; these correspond to locations approximately 2.4, 3.9, and 5.1 mm posterior to the bregma. To better illustrate the signal differences between groups, an expanded comparison of these slices with reference is provided in the three left columns of Figure 4A. Histological analysis was performed in brain slices following perfusion. The decrease in the HV visible on slices was visually correlated with drastic neuronal damage as shown in the Nissl-stained slices (Fig. 4B). We checked the region-specific patterns of neuronal damage within the anterior and posterior hippocampi. Nissl staining confirmed the extent of neuronal cell damage and the prominent loss of neurons in the CA1, CA3, and DG subregions (Fig. 4B). The volumetric analysis also confirmed the significant hippocampal atrophy, with an average reduction in the size of above 20% (Fig. 5Aa). The T2 signal intensity represented as T2 relaxation time was similar between control and soman-exposed groups (Fig. 5Ab).

A detailed analysis of the soman-induced decrease in the total HV revealed that the effects of soman exposure differed between the posterior or anterior and medial hippocampi, which was relatively intact with marginal loss in its volume (Fig. 6A). The extent of reduction of the left and right posterior HVs were smaller compared with those of the left and right anterior hippocampus. By contrast, T2 relaxation times across all HVs were similar (Fig. 6B), indicating the volume loss is related to long-lasting neuronal tissue atrophy in the hippocampus. Overall, these results show that soman exposure affects the anterior and posterior hippocampi much greater than the medial hippocampus. Visually, significant bilateral lesions were observed in the hippocampus, with a prominent expansion of the CSF, particularly in the more anterior sections. Asymmetric effects of soman exposure on anterior versus posterior HVs were evident from the MRI scans (Figs. 3 and 4A).

Soman-induced long-term morphological alterations in extrahippocampal brain regions

To determine permanent anatomical changes in the thalamus, similar procedures were employed to those for the hippocampus. The same three slices were analyzed for the thalamus (Fig. 4A). The voxel-based morphometry analysis was performed in slices covering the brain regions, including the thalamus. Overall, thalamic volumes (TVs) were similar between the two groups overall, and in each individual section (Fig. 5Ba). T2 relaxation times were also similar in both groups overall and in each of the three sections (Fig. 5Bb). Moreover, marked changes in tissue composition or size could not be observed in the MR images. Exposure to NAs may cause neuronal damage in a brain region-specific manner.

Soman-induced long-term changes in ventricles

Given the striking visible ventriculomegaly in the soman-exposed group, the LV analysis was conducted to quantify the extent of CSF expansion. Similar procedures were employed to those for the hippocampus and thalamus, but for the corresponding rightmost slice of Figure 4. Quantification of the volume and signal intensity showed that the LV volume was significantly increased in the soman-exposed group (Fig. 7A). T2 relaxation times were also significantly increased in the soman-exposed group, corresponding to a long-term T2 hyperintensity (Fig. 7B).

Soman-induced long-term spatial memory deficits

We have assessed the impairments in hippocampus-dependent spatial learning and memory around 90 days after exposure to soman. The performance of control and soman-exposed rats was tested in the Morris water maze, which is a widely used behavioral task for evaluating the ability of a rodent to learn to navigate a circular pool using distal cues to locate a hidden escape platform. The performance of animals in acquisition of spatial memory (session 1), memory retrieval (session 2), and memory retention (session 3) tasks were evaluated in both groups (Fig. 8A). Animals in both groups learned to locate the position of the submerged platform during training sessions. A two-way repeated-measures ANOVA showed that there were significant differences in latencies between the two groups ($P < 0.01$). Furthermore, progressive decreases in times to reach the platform over training sessions were seen in both groups. However, during the first session in the maze on days 1 to 7, soman-exposed rats required a significantly greater escape time over the course of seven

trials (Fig. 8B), implying reduced ability for acquisition of spatial memory. Control but not soman-exposed animals showed reductions in path lengths taken to escape to the platform. Analyses of data from the memory retrieval test performed 24 h after the last learning session demonstrated drastically impaired ability in soman-exposed rats as compared with control rats (Fig. 8C). Soman-exposed rats displayed limited memory or familiarity for the platform quadrant in the probe test (Fig. 8C), indicating memory retrieval dysfunction in these rats. Finally, the ability to recall the memory in the second probe test also showed clear differences between control and soman-exposed groups (Fig. 8D). Soman-exposed rats showed significantly reduced time around the platform area in the quadrant in the second probe test conducted on day 8 days after the last training. Thus, soman-exposed rats show reduced ability for acquisition of spatial memory, memory retrieval, and spatial memory retention in the water maze test.

Soman-induced long-term histological neurodegeneration in the hippocampus

In view of the major atrophy and decrease in a total volume reduction of the hippocampus in soman-exposed rats, we next performed histological analysis of neuronal damage and neurodegeneration. To determine whether soman exposure produced permanent neuronal damage and neurodegeneration, we measured the total number of principal neurons and inhibitory interneurons in the hippocampus using immunostaining and the neurostereology technique. We analyzed principal neurons through quantification of neurons positive for NeuN (a nuclear marker of mature neurons) in different subfields of the hippocampus (Fig. 9A). Soman-exposed rats exhibited a noticeable decrease in NeuN⁺ principal neurons than control rats in the CA1, CA3, and DG subfields (Fig. 9A). Since these neurons are checked around 90 days after exposure to soman, the results suggest that acute exposure to soman can trigger a long-lasting increase in neuronal damage and cell loss in the hippocampus. To quantify the extent of a long-term effect of soman exposure on hippocampal cell numbers, we measured the absolute total number of NeuN⁺ principal neurons in the hippocampus in animals belonging to both control and soman-exposed groups. The unbiased stereological analysis of total cell numbers revealed that soman-exposed animals exhibited significant (~40%) hippocampal cell loss in principal neurons ($P < 0.01$; Fig. 9E). The increase in neurodegeneration was significant for the entire hippocampus ($P < 0.001$, Fig. 9I). We also analyzed neurodegeneration of interneurons through quantification of neurons positive for PV (a marker of GABAergic inhibitory interneurons) in the entire hippocampus (Fig. 9B). The stereological analyses provided a measure of a long-lasting loss of PV⁺ interneurons (Fig. 9F), indicating the significant neurodegeneration of inhibitory interneurons in the whole hippocampus. Percent analyses demonstrated a significant increase in the extent of interneuron loss across the entire hippocampus of soman-exposed rats, in comparison with control rats (Fig. 9J).

Soman-induced long-term histological neuroinflammation in the hippocampus

NAs can cause massive neuroinflammation with hypertrophy of astrocytes and activation of microglial responses. IHC staining for glial fibrillary acidic protein (GFAP) shows an increased occurrence of astrocytes with hypertrophy in soman-exposed rats, in comparison with control rats (Fig. 9C). Therefore, we measured the area fraction of GFAP⁺ astrocyte elements in different subfields of the hippocampus. This quantification revealed significantly

enhanced astrocyte hypertrophy in the hippocampus of soman-exposed rats (Fig. 9G). The overall increase in inflammatory response was ~30% for the entire hippocampus ($P < 0.001$, Fig. 9K). IHC characterization using the IBA1 antibody revealed an increased occurrence of activated microglia displaying enlarged soma and fewer processes in soman-exposed rats (Fig. 9D). By contrast, control rats showed mostly ramified or resting microglia exhibiting very extensive fine processes (Fig. 9D). Hence, we quantified the area fraction of IBA1⁺ microglial elements in the hippocampus. This measurement shows significantly decreased occurrence of ramified or resting microglia in soman-exposed than control rats (Fig. 9H). The percentage calculation showed that, compared with microglia in control rats, microglia in soman-exposed animals displayed a 60% greater activation response (Fig. 9L).

Correlations between MRI-based HV loss and histological neurodegeneration and neuroinflammation

A longitudinal study design combining volumetric and histological measurement in the same animals enabled us to probe in detail the potential links between the HV and histological neurodegeneration. The MRI signals were significantly correlated ($r = 0.64\text{--}0.70$, $P < 0.001$) with histological neurodegeneration (NeuN and PV) in the hippocampus of the soman-exposed group (Fig. 10A and B). Notably, animals that had the greatest loss of principal neurons and interneurons at 90 days after soman exposure exhibited the most pronounced decrease in the HV (Fig. 10A, B, E, and F). In addition, the MRI signals were significantly correlated ($r = 0.65\text{--}0.70$, $P < 0.001$) with histological neuroinflammation (GFAP and IBA1) in the hippocampus of the soman-exposed group (Fig. 10C and D). There was a strong correlation between the histological parameters of neuroinflammation and total HV (Fig. 10C, D, G, and H).

Correlations between MRI-based HV loss and behavioral spatial memory deficits

A direct correlation analysis of outcomes from volumetric MRI and behavioral measurements in the same animals provide the potential links between hippocampal damage and performance in a hippocampus-dependent spatial memory task at 90 days following soman exposure. We found a significant correlation between the total HV and performance in the Morris water maze task (escape latencies in the first session) on day 5 (Fig. 11A) ($r = 0.64$, $P < 0.01$). Thus, animals that had a smaller HV were also more impaired in the spatial memory task. Remarkably, there was also a significant correlation between the total HV and performance in the spatial memory task on day 15 (Fig. 11B) ($r = 0.74$, $P < 0.01$). This suggests that animals with a smaller HV are more deficient in the spatial memory 1 week after a successful learning curve in the water maze.

Discussion

The main finding of this MRI study is the identification of chronic morphological biomarkers of nerve agent exposure, which is associated with long-lasting HV loss, atrophy, and ventricular enlargement and T2 hyperintensity in a subregion-specific pattern. The hippocampal atrophy is directly related to the massive neurodegeneration of principal neurons and interneurons and persistent microglial-mediated neuroinflammation in the hippocampus. Critical to these changes is that there are significant changes in learning and

memory performances in soman-exposed rats as compared with control animals both during acquisition of spatial memory and retrieval or recall of the spatial acquisition memory. In addition, a comparison of spatial memory and hippocampal atrophy changes after soman exposure revealed a direct relationship between cognitive performance and HV. These data demonstrate that HV loss and atrophy are highly consistent with soman-induced memory deficits and can act as chronic biomarkers for estimating the risk of long-term neurological memory impairments after acute nerve agent exposure. Given the large sample size and dynamic MRI scanning modality, these outcomes have a greater predictive power to characterize the chronic risk of soman-induced neurological dysfunction.

Long-term brain structural changes

The global BV is similar between the control and exposed cohorts, indicating that significant changes in the overall BV could not be discerned between groups. This result is consistent with human studies related to acute sarin exposure.³⁴ Since CSF volume is included in this measurement, this volume indicates the total space occupied by a completely solid brain, rather than purely the volume of grey and white matter. Thus, this result indicates that overall atrophy or enlargement of the brain in the skull has not occurred, but is not able to distinguish the extent of tissue loss. As seen in specific regions, atrophy of brain tissue can be offset by CSF expansion. A further study into specific brain tissue volume may be valuable but would be difficult to perform given the large sample of animals and the additionally time-consuming nature of separating the CSF during ROI. The similarity in BVs, however, has an additional value of reducing the attributability of region-specific volumetric differences to naturally unequal brain sizes between groups. There has been a limited study incorporating the full BV of rats using MRI, in part owing to a small size of the rat brain and increased postmortem accessibility compared with human brains. Nonetheless, a previous study has demonstrated that *in vivo* imaging can accurately reflect the TBVs in rats.⁵⁰ Overall BVs of the control group in this study align similarly with published MRI reports^{50,51}, which further supports our methodology.

A decrease in the HV

HVs of the control group align similarly with those MRI studies from literature.⁵¹⁻⁵³ The reduced HV in the OP-intoxicated group compared with the control group indicates effectively permanent tissue loss, especially in the posterior and anterior sections. This was an expected outcome since hippocampal atrophy has been shown to occur within 21 days of DFP intoxication.⁴⁹ Significant lesions were observed on the lateral ends of the hippocampus, corresponding to the CA3, the hippocampal region known for some of the greatest neurodegeneration.⁵⁴ Furthermore, T2 relaxation times of the control group were consistent with the literature values.⁵⁵ T2 relaxation times were similar in OP-exposed rats to the control. The general acute T2 hyperintensity of the hippocampus is known to diminish in weeks.⁴⁹ Thus, overall hippocampal hyperintensity no longer becomes an effective metric for long-term *in vivo* monitoring and fails to reflect the widespread neurodegeneration observed in histological data. Specific focus on observing small hyperintense areas of lesion, however, in regions, such as the CA3, better reflects the presence of chronic neuropathology. The decrease in the HV is positively correlated with histological outcomes of neurodegeneration and neuroinflammation in the hippocampal slices from the soman-

exposed group. The stereological analysis of the extent of neuronal cell loss in the hippocampus provided a direct approach to assess neurodegeneration after 90 days of soman exposure. The reduction in the HV correlated with increased neuroinflammation in the hippocampal slices, as evident from a marked increase in astrogliosis and microgliosis in the soman-exposed group. It is likely that soman exposure is associated with persistent neuroinflammation in the hippocampus and might be linked to neuronal damage, neuronal cell loss, and atrophy in the hippocampus. However, a time-course examination of temporal morphological and memory function changes is beyond the scope of this study, which is intended to capture the long-term impact of nerve agent exposure. There are few previous studies on the temporospatial progression of neuroinflammation in a rat model of DFP intoxication.⁵⁶⁻⁵⁸ These reports are consistent with our results on the persistent, long-term neuropathological dysfunction in the soman model. However, potential differences in temporal aspects of neuroinflammation, such as its persistence for 3 months postexposure to soman. It is likely that soman caused such long-term neuroinflammation because it is a more potent neurotoxic agent and produces massive short- and long-term neuronal damage, as evident from previous studies.^{57, 58} Such inflammatory response-related neuronal damage may trigger a vicious cycle that appears to reflect as long-lasting inflammation in the soman model.

The thalamus and other brain regions

To our knowledge, limited *in vivo* estimates of the TV, as well as comparisons of the TV in soman-exposed animals, have been published in the literature. The TV was similar in control and soman-exposed animals, indicating either minimal or harder-to-distinguish tissue loss compared with the hippocampus. However, these MRI outcomes did not correlate with the histological damage in the thalamus, as observed with sensitive IHC markers. T2 relaxation times of the control group were comparable with previous reports.^{59, 60} Thalamic T2 relaxation times were not significantly changed in the long term owing to soman exposure. Given the pilot nature of this measurement as applied to the soman-exposed group, T2 imaging for complete TVs does not appear to be a feasible chronic monitoring target. Using a higher field strength scanner outside of the clinical range and altering acquisition parameters to boost contrast in relatively hypointense regions like the thalamus could possibly reveal specific lesion sites. Under diffusion- rather than T2-weighted imaging, transient increases in apparent diffusion coefficient decrease to statistical nonsignificance within 1 month of exposure to DFP.⁴⁹

Previous studies of soman neuropathology observed acute decreases in T2 relaxation and diffusion-weighted imaging apparent diffusion in the hippocampus and thalamus, and prolonged (1 week) decreases in the amygdala and PC.^{47, 61} Other OP models observed increased T2 relaxation times in the thalamus and parietotemporal cortices 3 h after exposure to the OP paraoxon.⁴⁸ The additional regions specified in models such as these represent additional targets for long-term MRI study. In particular, the PC is another significant region to explore in future studies. Visually, this region displayed hyperintensity in this study, but the exact extent was not quantified. The PC is known to display extensive neurodegeneration and calcification months after DFP exposure.^{54, 62} Future investigation with additional focus on the PC could offer new insights on further potential biomarkers for

soman-induced neuropathology. Further studies are in progress to investigate the MRI defects in the amygdala and other extrahippocampal regions. In a micro-CT scan study in DFP-exposed rats, OP intoxication can produce neurological damage via seizure-independent mechanisms.⁵⁶ In soman-intoxicated guinea pigs, there is a long-lasting increase in a T2-weighted signal intensities in the PC and other areas, indicating a structural PC damage induced by acute exposure to soman.⁶³ These reports are consistent with our MRI observations in the soman-exposed model.

Enlargement of ventricles

The LV volume was significantly (almost two-fold) increased in soman-exposed animals. Such increase in ventricle volume was expected, as shorter-term longitudinal studies have observed a gradual increase in the ventricular volume over the scale of weeks after DFP.⁴⁹ The increased CSF volume is similar to the lost HV, suggesting compensatory fluid expansion secondary to regional brain atrophy. The high contrast of the ventricles provides for easy visualization of the volume and can be an effective long-term tool for indirectly gauging tissue loss in other areas. Interestingly, T2 relaxation times for the LV increased in the soman-exposed group. This suggests a change in composition in this region, such as increased CSF density or overlapping scarring of the underlying tissue. These findings are consistent with previous studies in a rat model of DFP intoxication.⁴⁹ Although there are a few studies using MRI to evaluate soman-induced enlargement of the ventricle,⁶⁴ there are some reports on soman-induced loss of neurons and enlargement of ventricles using neuropathology assessments.^{65,66} These reports strongly support our findings on the enlargement of ventricles, which appears to be a hallmark neuropathological feature of soman exposure. Thus, MRI represents a promising noninvasive tool to identify this biomarker, assess its progression longitudinally, and to evaluate the efficacy of a neuroprotective treatment.

In conclusion, these results demonstrate chronic MRI lesions and volumetric abnormalities in the hippocampus and other key areas of the brain in the soman model of nerve agent intoxication. Moreover, these long-lasting changes are positively correlated with the histological markers of neurodegeneration and neuroinflammation in the hippocampus, and can predict the special learning and memory dysfunction. HV loss and atrophy are linked to soman-induced memory deficits and hence may be a potential biomarker for predicting the risk of long-term development of neurological dysfunction after nerve agent exposure. Together, these findings support the application of MRI as a sensitive and versatile *in vivo* neuroimaging tool to gain early insight into the anatomic extent and spatial progression in morphological defects in the brain after nerve agent exposure.

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References:

1. McDonough JH Jr. and Shih TM. 1997 Neuropharmacological mechanisms of nerve agent-induced seizure and neuropathology. *Neuroscience and biobehavioral reviews*. 21: 559–579. [PubMed: 9353792]
2. Chen Y 2012 Organophosphate-induced brain damage: mechanisms, neuropsychiatric and neurological consequences, and potential therapeutic strategies. *Neurotoxicology*. 33: 391–400. [PubMed: 22498093]
3. Henretig FM, Kirk MA and McKay CA Jr. 2019 Hazardous Chemical Emergencies and Poisonings. *N Engl J Med*. 380: 1638–1655. [PubMed: 31018070]
4. Morita H, Yanagisawa N, Nakajima T, et al. 1995 Sarin poisoning in Matsumoto, Japan. *Lancet*. 346: 290–293. [PubMed: 7630252]
5. Suzuki HR, Solursh M and Baldwin HS. 1995 Relationship between fibronectin expression during gastrulation and heart formation in the rat embryo. *Dev Dyn*. 204: 259–277. [PubMed: 8573718]
6. Moshiri M, Darchini-Maragheh E and Balali-Mood M. 2012 Advances in toxicology and medical treatment of chemical warfare nerve agents. *Daru*. 20: 81. [PubMed: 23351280]
7. Abou-Donia MB, Siracuse B, Gupta N, et al. 2016 Sarin (GB, O-isopropyl methylphosphonofluoridate) neurotoxicity: critical review. *Crit Rev Toxicol*. 46: 845–875. [PubMed: 27705071]
8. Reddy DS and Colman E. 2017 A comparative toxidrome analysis of human organophosphate and nerve agent poisonings using social media. *Clin Transl Sci*. 10: 225–230. [PubMed: 28238224]
9. Ciotto GR 2018 Toxidrome Recognition in Chemical-Weapons Attacks. *N Engl J Med*. 378: 1611–1620. [PubMed: 29694809]
10. Apland JP, Figueiredo TH, Qashu F, et al. 2010 Higher susceptibility of the ventral versus the dorsal hippocampus and the posteroventral versus anterodorsal amygdala to soman-induced neuropathology. *Neurotoxicology*. 31: 485–492. [PubMed: 20570628]
11. de Araujo Furtado M, Zheng A, Sedigh-Sarvestani M, et al. 2009 Analyzing large data sets acquired through telemetry from rats exposed to organophosphorous compounds: an EEG study. *J Neurosci Methods*. 184: 176–183. [PubMed: 19632275]
12. Figueiredo TH, Apland JP, Braga MFM, et al. 2018 Acute and long-term consequences of exposure to organophosphate nerve agents in humans. *Epilepsia*. 59 Suppl 2: 92–99.
13. Shih TM and McDonough JH Jr. 1997 Neurochemical mechanisms in soman-induced seizures. *Journal of applied toxicology : JAT*. 17: 255–264. [PubMed: 9285539]
14. Marrero-Rosado B, de Araujo Furtado M, Schultz CR, et al. 2018 Soman-induced status epilepticus, epileptogenesis, and neuropathology in carboxylesterase knockout mice treated with midazolam. *Epilepsia*. 59: 2206–2218. [PubMed: 30368799]
15. McDonough JH, McMonagle JD and Shih TM. 2010 Time-dependent reduction in the anticonvulsant effectiveness of diazepam against soman-induced seizures in guinea pigs. *Drug and chemical toxicology*. 33: 279–283. [PubMed: 20429808]
16. Apland JP, Aroniadou-Anderjaska V, Figueiredo TH, et al. 2014 The limitations of diazepam as a treatment for nerve agent-induced seizures and neuropathology in rats: comparison with UBP302. *J Pharmacol Exp Ther*. 351: 359–372. [PubMed: 25157087]
17. Reddy SD and Reddy DS. 2015 Midazolam as an anticonvulsant antidote for organophosphate intoxication-A pharmacotherapeutic appraisal. *Epilepsia*. 56: 813–821. [PubMed: 26032507]
18. Nishiwaki Y, Maekawa K, Ogawa Y, et al. 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. [PubMed: 11713003]
19. Ohtani T, Iwanami A, Kasai K, et al. 2004 Post-traumatic stress disorder symptoms in victims of Tokyo subway attack: a 5-year follow-up study. *Psychiatry Clin Neurosci*. 58: 624–629. [PubMed: 15601387]
20. Monograph NTP. 2019 Systematic Review of Long-term Neurological Effects Following Acute Exposure to Sarin. National Toxicology Program, U.S. Department of Health and Human Services pp1–177.

21. Murata K, Araki S, Yokoyama K, et al. 1997 Asymptomatic sequelae to acute sarin poisoning in the central and autonomic nervous system 6 months after the Tokyo subway attack. *J Neurol.* 244: 601–606. [PubMed: 9402534]
22. Savage EP, Keefe TJ, Mounce LM, et al. 1988 Chronic neurological sequelae of acute organophosphate pesticide poisoning. *Arch Environ Health.* 43: 38–45. [PubMed: 3355242]
23. Yanagisawa N, Morita H and Nakajima T. 2006 Sarin experiences in Japan: acute toxicity and long-term effects. *J Neurol Sci.* 249: 76–85. [PubMed: 16962140]
24. Reddy DS 2016 Neurosteroids for the potential protection of humans against organophosphate toxicity. *Ann N Y Acad Sci.* 1378: 25–32. [PubMed: 27450921]
25. Lumley L, Miller D, Muse WT, et al. 2019 Neurosteroid and benzodiazepine combination therapy reduces status epilepticus and long-term effects of whole-body sarin exposure in rats. *Epilepsia Open.* 4: 382–396. [PubMed: 31440720]
26. Reddy DS 2019 Mechanism-based novel antidotes for organophosphate neurotoxicity. *Current Opinion in Toxicology.* 14:35–45. [PubMed: 32856007]
27. Reddy SD, Younus I, Sridhar V, et al. 2019 Neuroimaging Biomarkers of Experimental Epileptogenesis and Refractory Epilepsy. *Int J Mol Sci.* 20.
28. Bernasconi A, Bernasconi N, Caramanos Z, et al. 2000 T2 relaxometry can lateralize mesial temporal lobe epilepsy in patients with normal MRI. *Neuroimage.* 12: 739–746. [PubMed: 11112405]
29. Hobson BA, Rowland DJ, Supasai S, et al. 2018 A magnetic resonance imaging study of early brain injury in a rat model of acute DFP intoxication. *Neurotoxicology.* 66: 170–178. [PubMed: 29183789]
30. Wang H, Huang Y, Coman D, et al. 2017 Network evolution in mesial temporal lobe epilepsy revealed by diffusion tensor imaging. *Epilepsia.* 58: 824–834. [PubMed: 28378878]
31. Testylier G, Lahrech H, Montigon O, et al. 2007 Cerebral edema induced in mice by a convulsive dose of soman. Evaluation through diffusion-weighted magnetic resonance imaging and histology. *Toxicology and applied pharmacology.* 220: 125–137. [PubMed: 17350063]
32. Lemercier G, Carpentier P, Sentenac-Roumanou H, et al. 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. [PubMed: 6637396]
33. Harrison PK, Sheridan RD, Green AC, et al. 2004 A guinea pig hippocampal slice model of organophosphate-induced seizure activity. *J Pharmacol Exp Ther.* 310: 678–686. [PubMed: 15031302]
34. Yamasue H, Abe O, Kasai K, et al. 2007 Human brain structural change related to acute single exposure to sarin. *Ann Neurol.* 61: 37–46. [PubMed: 17187377]
35. Kim YB, Hur GH, Shin S, et al. 1999 Organophosphate-induced brain injuries: delayed apoptosis mediated by nitric oxide. *Environ Toxicol Pharmacol.* 7: 147–152. [PubMed: 21781920]
36. Figueiredo TH, Aroniadou-Anderjaska V, Qashu F, et al. 2011 Neuroprotective efficacy of caramiphen against soman and mechanisms of its action. *Br J Pharmacol.* 164: 1495–1505. [PubMed: 21486285]
37. Wu X, Kuruba R and Reddy DS. 2018 Midazolam-Resistant Seizures and Brain Injury following Acute Intoxication of Diisopropylfluorophosphate, an Organophosphate Pesticide and Surrogate for Nerve Agents. *J Pharmacol Exp Ther.* 367: 302–321. [PubMed: 30115757]
38. Racine RJ 1972 Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroencephalogr Clin Neurophysiol.* 32: 281–294. [PubMed: 4110397]
39. de Graaf RA, Brown PB, McIntyre S, et al. 2006 High magnetic field water and metabolite proton T1 and T2 relaxation in rat brain in vivo. *Magn Reson Med.* 56: 386–394. [PubMed: 16767752]
40. Nie B, Chen K, Zhao S, et al. 2013 A rat brain MRI template with digital stereotaxic atlas of fine anatomical delineations in paxinos space and its automated application in voxel-wise analysis. *Hum Brain Mapp.* 34: 1306–1318. [PubMed: 22287270]
41. Paxinos G and Watson C. 2007 The rat brain in stereotaxic coordinates (6th Edition) Academic Press New York.
42. Winston GP, Vos SB, Burdett JL, et al. 2017 Automated T2 relaxometry of the hippocampus for temporal lobe epilepsy. *Epilepsia.* 58: 1645–1652. [PubMed: 28699215]

43. Golub VM, Brewer J, Wu X, et al. 2015 Neurostereology protocol for unbiased quantification of neuronal injury and neurodegeneration. *Frontiers in Aging Neuroscience*. 7: 196. [PubMed: 26582988]
44. Kuruba R, Wu X and Reddy DS (2018). Benzodiazepine-refractory status epilepticus, neuroinflammation, and interneuron neurodegeneration after acute organophosphate intoxication. *Biochim Biophys Acta* 1864(9 Pt B): 2845–2858.
45. Morris R 1984 Developments of a water-maze procedure for studying spatial learning in the rat. *J Neurosci Methods*. 11: 47–60. [PubMed: 6471907]
46. Hattiangady B, Kuruba R and Shetty AK. 2011 Acute Seizures in Old Age Leads to a Greater Loss of CA1 Pyramidal Neurons, an Increased Propensity for Developing Chronic TLE and a Severe Cognitive Dysfunction. *Aging Dis*. 2: 1–17. [PubMed: 21339903]
47. Bhagat YA, Obenaus A, Hamilton MG, et al. 2005 Neuroprotection from soman-induced seizures in the rodent: evaluation with diffusion- and T2-weighted magnetic resonance imaging. *Neurotoxicology*. 26: 1001–1013. [PubMed: 15982742]
48. Shrot S, Tauber M, Shiyovich A, et al. 2015 Early brain magnetic resonance imaging can predict short and long-term outcomes after organophosphate poisoning in a rat model. *Neurotoxicology*. 48: 206–216. [PubMed: 25912464]
49. Hobson BA, Siso S, Rowland DJ, et al. 2017 From the Cover: Magnetic Resonance Imaging Reveals Progressive Brain Injury in Rats Acutely Intoxicated With Diisopropylfluorophosphate. *Toxicol Sci*. 157: 342–353. [PubMed: 28329842]
50. Oguz I, Yaxley R, Budin F, et al. 2013 Comparison of magnetic resonance imaging in live versus post mortem rat brains. *PLoS One*. 8: e71027. [PubMed: 23967148]
51. Hamezah HS, Durani LW, Ibrahim NF, et al. 2017 Volumetric changes in the aging rat brain and its impact on cognitive and locomotor functions. *Exp Gerontol*. 99: 69–79. [PubMed: 28918364]
52. Lee T, Jarome T, Li SJ, et al. 2009 Chronic stress selectively reduces hippocampal volume in rats: a longitudinal magnetic resonance imaging study. *Neuroreport*. 20: 1554–1558. [PubMed: 19858767]
53. Rahman MM, Callaghan CK, Kerskens CM, et al. 2016 Early hippocampal volume loss as a marker of eventual memory deficits caused by repeated stress. *Sci Rep*. 6: 29127. [PubMed: 27374165]
54. Kadar T, Shapira S, Cohen G, Sahar R, Alkalay D, Raveh L (1995). Sarin-induced neuropathology in rats. *Human Exp Toxicol* 14: 252–259.
55. Jackson GD, Connelly A, Duncan JS, et al. 1993 Detection of hippocampal pathology in intractable partial epilepsy: increased sensitivity with quantitative magnetic resonance T2 relaxometry. *Neurology*. 43: 1793–1799. [PubMed: 8414034]
56. Gonzalez EA, Rindy AC, Guignet MA, et al. 2020 The chemical convulsant diisopropylfluorophosphate (DFP) causes persistent neuropathology in adult male rats independent of seizure activity. *Archives of toxicology*.
57. Flannery BM, Bruun DA, Rowland DJ, et al. 2016 Persistent neuroinflammation and cognitive impairment in a rat model of acute diisopropylfluorophosphate intoxication. *J Neuroinflammation*. 13: 267. [PubMed: 27733171]
58. Guignet M, Dhakal K, Flannery BM, et al. 2020 Persistent behavior deficits, neuroinflammation, and oxidative stress in a rat model of acute organophosphate intoxication. *Neurobiol Dis*. 133: 104431. [PubMed: 30905768]
59. Fabene PF, Marzola P, Sbarbati A, et al. 2003 Magnetic resonance imaging of changes elicited by status epilepticus in the rat brain: diffusion-weighted and T2-weighted images, regional blood volume maps, and direct correlation with tissue and cell damage. *Neuroimage*. 18: 375–389. [PubMed: 12595191]
60. de Graaf RA, Brown PB, McIntyre S, Nixon TW, Behar KL, Rothman DL (2006). High magnetic field water and metabolite proton T1 and T2 relaxation in rat brain in vivo. *Magnetic Resonance in Medicine* 56: 386–394. [PubMed: 16767752]
61. Bhagat YA, Obenaus A, Hamilton MG, et al. 2001 Magnetic resonance imaging predicts neuropathology from soman-mediated seizures in the rodent. *Neuroreport*. 12: 1481–1487. [PubMed: 11388434]

62. Zimmer LA, Ennis M, and Shipley MT (1997). Soman-induced seizures rapidly activate astrocytes and microglia in discrete brain regions. *J Comp Neurol* 378: 482–492. [PubMed: 9034905]
63. Gullapalli RP, Aracava Y, Zhuo J, et al. 2010 Magnetic resonance imaging reveals that galantamine prevents structural brain damage induced by an acute exposure of guinea pigs to soman. *Neurotoxicology*. 31: 67–76. [PubMed: 19782102]
64. Carpentier P, Testylier G, Dorandeu F, et al. 2008 Hyperosmolar treatment of soman-induced brain lesions in mice: evaluation of the effects through diffusion-weighted magnetic resonance imaging and through histology. *Toxicology*. 253: 97–103. [PubMed: 18824071]
65. Tryphonas L and Clement JG. 1995 Histomorphogenesis of soman-induced encephalocardiomyopathy in Sprague-Dawley rats. *Toxicol Pathol*. 23: 393–409. [PubMed: 7659961]
66. Carpentier P, Foquin A and Dorandeu F. 2010 Hypertonic mannitol in mice poisoned by a convulsive dose of soman: antilethal activity without neuroprotection. *Toxicology*. 268: 78–88. [PubMed: 20003915]

Figure 1.

Experimental protocol for the acute soman exposure model. (A) Rats were exposed to soman (154 $\mu\text{g/kg}$, 1.4- LD_{50}) by a single subcutaneous injection, as per the previously published protocol (see the Materials and methods section). The HI-6 (125 mg/kg, i.p.) was administered 30 min prior to soman to increase the survival rate. Within 1 min of soman exposure, rats were treated with atropine methylnitrate (2 mg/kg, i.m.) to minimize peripheral toxic effects. The anticonvulsant midazolam (2 mg/kg, i.m.) was administered at 40 min after soman exposure to control seizures and improve survival. Animals were monitored for 24 h postexposure for behavioral seizure activity. MRI scanning was performed around 90 days after exposure to soman. Four to six rats were scanned in a single day at the Texas A&M Imaging facility. Memory function was evaluated by the Morris water maze test 90 days after exposure to soman. Since the Morris water maze is a 7-day test, it was started around day 93. Then, animals were perfused to fix the brain for histopathological examination. All soman exposure studies were conducted at the MRIGlobal facility (Kansas City, MO). (B) Acute behavioral seizure activity after soman exposure in rats during the 24-h monitoring period. Behavioral seizures were monitored by video recording and scored on the Racine scale. (C) A representative EEG trace of seizure activity in one rat with implanted EEG electrodes following soman exposure and after midazolam treatment. The data in panel B represents the mean of the group seizure scores ($n = 10$ for the control group and $n = 22$ for the soman-exposed group).

Figure 2.

MRI T2-weighted images of full sagittal sections in control and soman-exposed rats. MRI scans were acquired around 90 days after exposure to soman. (A) Representative sagittal sections of randomly selected control (top panel) and soman-exposed (bottom panel) animals. The T2-weighted 1.5-mm thick sagittal slices have TE/TR of 37/2600 milliseconds. Overall, there were no major anatomical differences between control and soman-exposed groups. (B) TBVs were similar between the two groups. All data represents the mean $\pm$ SEM ($n = 10$ for the control group and $n = 17$ for the soman-exposed group). Scale bar = 10 mm.

Figure 3.

Images of full coronal sections of control and soman-exposed rats. Representative coronal sections of randomly selected control (left) and soman-exposed (right) animals. Sections span from posterior (top left) to anterior (bottom right). The T2-weighted 1.5-mm thick coronal slices have echo time/repetition time of 123/3610 milliseconds. The concentric lines on the bottom right sagittal images represent the special orientation of coronal slices. Yellow arrows signify the areas of pathological abnormalities in a soman-exposed animal. Significant bilateral lesions and fluid expansion are visible in the hippocampus (yellow arrows) and LVs (yellow arrows). Scale bar = 10 mm.

Figure 4.

MRI T2-weighted comparative changes in the hippocampus, thalamus, and ventricles between control and soman-exposed rats. (A) Representative MR images from the control and soman-exposed groups. The anatomical diagram (top) from the Paxinos and Watson's *Rat Brain Atlas* compared with respective coronal sections of randomly selected control (middle) and soman-exposed (bottom) rats. Yellow arrows signify areas of pathological abnormalities in the soman-exposed animal. The MRI scans show bilateral hippocampal atrophy and lesions (yellow arrows), while fluid expansion is also visible in the hippocampus and LVs (yellow arrows). Images were selected from the control ($n = 10$) and soman-exposed ($n = 17$) groups. Scale bar = 10 mm. (B) A histological brain section showing Nissl-stained pattern depicting neuronal cell loss or damage in hippocampal subfields CA1, CA3, dentate gyrus (DG), and DG regions in the soman-exposed group. Scale bar = 500 μm (top panel; hippocampus) and 150 μm (bottom three panels).

Figure 5.

Average volumes and T2 relaxation times of the hippocampus and thalamus in control and soman-exposed rats. (A) Soman-exposed animals showed drastically reduced HVs (Aa), but T2 relaxation times in the hippocampus (Ab) were similar between groups. (B) Soman-exposed animals showed similar thalamus volumes (Ba) and T2 relaxation times in the thalamus (Bb) between groups. All data represents the mean $\pm$ SEM ($n = 10$ for the control group and $n = 17$ for the soman-exposed group), and asterisks signify statistical significance (* $P < 0.01$ versus the control group).

Figure 6.

Comparative average volumes and T2 relaxation times of various brain regions in control and soman-exposed groups. (A) Brain tissue volumes were significantly different between groups in the posterior and anterior hippocampi, and ventricles. The HV loss shows an asymmetry between the posterior or anterior and medial hippocampi. The right and left posterior and anterior HV in exposed animals are significantly lower than in control animals. However, the extent of the volume loss in posterior hippocampi is significantly lower than in the anterior subregion in soman-exposed animals. (B) T2 relaxation times were similar between groups in all brain regions except cerebral ventricles, which exhibited heightened T2 signals owing to massive fluid expansion. All data represents the mean $\pm$ SEM ($n = 10$ for the control group and $n = 17$ for the soman-exposed group), and asterisks signify statistical significance (* $P < 0.01$ versus the control group).

Figure 7.

Average volumes and T2 relaxation times of the LVs in control and soman-exposed rats. (A) Soman-exposed animals showed drastically increased ventricular volumes. (B) T2 relaxation times for the ventricles were significantly higher in soman-exposed animals. All data represents the mean $\pm$ SEM ($n = 10$ for the control group and $n = 17$ for the soman-exposed group), and asterisks signify statistical significance ($*P < 0.01$ versus the control group).

Figure 8.

Long-term memory dysfunction in soman-exposed rats in the Morris water maze test. (A) A schematic illustration of the experimental sessions for acquisition of spatial memory and memory retention assessment in rats. (B) Control animals had significantly shorter latencies in the water maze as compared with soman-exposed rats across all days tested. (C) A probe test conducted 24 h after the last learning session revealed lesser memory retrieval performance in the soman-exposed group as these rats showed reduced dwell time in the platform quadrant. (D) Memory test conducted 8 days after the last learning session revealed significant soman-related deficits in memory recovery for the platform quadrant as these rats showed reduced dwell time in the platform quadrant. All data represents the mean $\pm$ SEM ($n = 10$ for the control group and $n = 17$ for the soman-exposed group), and asterisks signify statistical significance ($*P < 0.01$ versus the control group).

Figure 9.

Histological neuropathology in the brains of control and soman-exposed rats. (A–D) Representative histological sections of the hippocampus from control and soman-exposed rats illustrating neurodegeneration and neuroinflammation patterns around 90 days after exposure to soman. Scale bar = 500 μ m (hippocampus) or 150 μ m (CA1, CA2, and DG). (A, E, and I) NeuN⁺ immunostaining of principal neurons in hippocampal subfields CA1, CA3, and DG (A), absolute total NeuN⁺ cell numbers in the total hippocampus (E), and the extent of NeuN⁺ cell loss in the total hippocampus (I). (B, F, and J) PV⁺ immunostaining of inhibitory interneurons in hippocampal subfields CA1, CA3 and DG (B), absolute total NeuN⁺ cell numbers in the total hippocampus (F), and the extent of NeuN⁺ cell loss in the total hippocampus (J). (C, G, and K) GFAP⁺ immunostaining of neuroinflammatory astrogliosis in hippocampal subfields (C), the area fraction (AF) density of GFAP⁺ astrogliosis in the total hippocampus (G), and the percent inflammatory response in the total hippocampus (K). (D, H, and L) IBA1⁺ immunostaining of neuroinflammatory microgliosis in hippocampal subfields (D), the area fraction density of IBA1⁺ microgliosis in the total hippocampus (H), and the percent inflammatory response in the total hippocampus (L). All data represents the mean $\pm$ SEM ($n = 10$ for the control group and $n = 10$ randomly selected from the soman group; $n = 17$), and asterisks signify statistical significance (* $P < 0.01$ versus the control group).

Figure 10.

Scatterplots illustrating the relationship between the MRI HV reduction and the histological neurodegenerative (A, B, E, and F) and neuroinflammatory (C, D, G, and H) changes in the hippocampus in control and soman-exposed groups. (A and E) The absolute NeuN⁺ principal cell numbers (A) and percent cell loss (E) in the hippocampus were positively correlated with the HV reduction in soman-exposed rats ($r = 0.68$ and $r = 0.69$, $P < 0.001$). (B and F) The absolute PV⁺ inhibitory interneuron numbers (B) and percent interneuron loss (F) in the hippocampus were significantly correlated with the HV reduction in soman-exposed rats ($r = 0.65$ and $r = 0.64$, $P < 0.001$). (C and G) The HV reduction in soman-exposed rats was significantly correlated to both GFAP⁺ astrogliosis neuroinflammation (C) and percent astrocytic neuroinflammation ($r = 0.70$ and $r = 0.70$, $P < 0.001$). (D and H) Both IBA1⁺ microgliosis neuroinflammation (D) and percent microglial neuroinflammation (H) were significantly correlated with the HV reduction in soman-exposed rats ($r = 0.69$, $P < 0.001$). $n = 10$ for the control group and $n = 10$ for the soman-exposed group).

Figure 11.

Correlation between long-term decrease in the MRI-measured HV and behavioral spatial memory deficit in soman-exposed rats. (A) Decrease in the HV, at 90 days postexposure, is significantly correlated with spatial memory acquisition ($r = 0.62$; $P < 0.01$, Pearson's correlation). (B) Animals with the lower HV have impaired ability for spatial memory retention ($r = 0.74$; $P < 0.01$); these animals perform more poorly in the memory recall on day 15 ($n = 10$ for the control group and $n = 10$ for the soman-exposed group).