Original Research

Delayed tezampanel and caramiphen treatment but not midazolam protects against long-term neuropathology after soman exposure

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Impact Statement

Nerve agents are the most dreaded chemical weapons of mass destruction due to their fast action, high lethality by an agonizing death, relatively simple synthesis, and easy transport and deployment. These agents have been used in wars, terrorist attacks, and as assassination tools. Acute exposure to nerve agents induces status epilepticus (SE), which can cause brain damage or death if not treated effectively. In this study, conducted in rats of an age that corresponds to the pediatric human population, we found that midazolam – the anticonvulsant that was recently approved by the Food and Drug Administration (FDA) for the treatment of nerve agent–induced SE – did not protect the brain against SE induced by the nerve agent soman, when administered with a delay after SE onset. In contrast, co-administration of tezampanel and caramiphen provided nearly full neuroprotection. These findings can be applicable to the treatment of prolonged SE of any etiology.

Abstract

Prolonged status epilepticus (SE) can cause brain damage; therefore, treatment must be administered promptly after seizure onset to limit SE duration and prevent neuropathology. Timely treatment of SE is not always feasible; this would be particularly true in a mass exposure to an SE-inducing agent such as a nerve agent. Therefore, the availability of anticonvulsant treatments that have neuroprotective efficacy even if administered with a delay after SE onset is an imperative. Here, we compared the long-term neuropathology resulting from acutely exposing 21-day-old male and female rats to the nerve agent soman, and treating them with midazolam (3 mg/kg) or co-administration of tezampanel (10 mg/kg) and caramiphen (50 mg/kg), at 1 h postexposure (~50 min after SE onset). Midazolam-treated rats had significant neuronal degeneration in limbic structures, mainly at one month postexposure, followed by neuronal loss in the basolateral amygdala and the CA1 hippocampal area. Neuronal loss resulted in significant amygdala and hippocampal atrophy, deteriorating from one to six months postexposure. Rats treated with tezampanel–caramiphen had no evidence of neuropathology, except for neuronal loss in the basolateral amygdala at the six-month timepoint. Anxiety was increased only in the midazolam-treated rats, at one, three, and six months postexposure. Spontaneous recurrent seizures appeared only in midazolam-treated rats, at three and six months postexposure in males and only at six months in females. These findings suggest that delayed treatment of nerve agent–induced SE with midazolam may result in long-lasting or permanent brain damage, while antiglutamatergic anticonvulsant treatment consisting of tezampanel and caramiphen may provide full neuroprotection.

Keywords: Nerve agents, soman, status epilepticus, midazolam, tezampanel, LY293558, caramiphen, neuroprotection

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Introduction

Prolonged status epilepticus (SE) of any etiology can cause brain damage or death,[1,2] making prompt and effective treatment an imperative. However, treatment cannot always be prompt. For example, in the event of a mass civilian or military exposure to a nerve agent, which can cause severe SE, it is unlikely that medical care can be promptly provided to all victims. Therefore, the availability of treatments that are effective in terminating SE and protecting against brain damage even if administered with a delay after SE onset is essential.

Seizures induced by nerve agent exposure are triggered by muscarinic receptor hyperstimulation, following the inhibition of acetylcholinesterase by the organophosphorus agent and the excessive elevation of acetylcholine in central

cholinergic synapses.[3,4] Muscarinic receptor hyperstimulation is central only in the initial stages of SE, whereas the ensuing glutamatergic hyperactivity strengthens and sustains seizures.[5–8] Therefore, one way to suppress seizures is to counteract glutamatergic hyperexcitation by enhancing GABAergic inhibition. Accordingly, the Food and Drug Administration (FDA) has approved the benzodiazepine diazepam and, recently, midazolam (MDZ) for the control of SE induced by nerve agent exposure. This is consistent with the common clinical use of a benzodiazepine as first-line treatment of SE regardless of the etiology.[1,9,10] By enhancing $GABA_A$ receptor-mediated inhibitory activity,[11,12] diazepam or MDZ can be effective in terminating SE and protecting against brain damage, as long as the benzodiazepine is administered shortly after SE onset. However, if treatment is delayed, SE may become refractory to benzodiazepines[1,13] or the cessation of SE by benzodiazepine administration is only transient; intense seizures reoccur[14] and require additional pharmacological interventions that will control SE and prevent brain damage or death. With regard to SE induced by nerve agents or other organophosphorus toxins, animal studies have shown that if diazepam is administered with a delay after SE onset, there is significant reoccurrence of seizures and little to no neuroprotection.[15–17]

Seizures can also be suppressed by directly counteracting glutamatergic hyperactivity using antagonists of specific glutamate receptor subtypes that play key roles in seizure generation and neuronal damage. We have found that tezampanel (LY293558; (3*S*,4*aR*,6*R*,8*aR*)-6-[2-(1(2)*H*-tetrazole-5-yl) ethyl]decahydroisoquinoline-3-carboxylic acid), an AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid)/GluK1 kainate receptor antagonist,[18] not only stops SE when administered with 1-h delay after exposure to the nerve agent soman (about 50 min after ongoing SE), but it also provides significant protection against brain damage.[19–22] Neuroprotection is further enhanced if LY293558 is given along with caramiphen (CRM),[16,23] an antimuscarinic compound which also antagonizes *N*-methyl-d-aspartate (NMDA) receptors.[24–27] We recently compared the antiseizure efficacy of LY293558 + CRM with that of MDZ, the benzodiazepine which – after its recent FDA approval – will gradually replace diazepam, as the preferred alternative for the control of nerve agent–induced SE. We conducted these experiments in 21-day-old rats (P21 rats) to obtain data that are relevant to the pediatric population, and administered the anticonvulsants with 1-h delay after soman exposure. We found that both MDZ and LY293558 + CRM promptly stopped the initial SE, but strong seizures reoccurred in the MDZ-treated group, resulting in a total duration of SE in this group significantly greater than the total duration of SE in the LY293558 + CRM group.[28] In this study, we investigated the neuropathological consequences of these treatments, up to six months post-soman exposure.

Materials and methods

Animals

Sprague–Dawley male and female rat pups (Charles River Laboratories, Wilmington, MA) were ordered and shipped separately, in groups of 10, each sex with a different surrogate mother. The rats were housed in an environmentally controlled room (20–23°C, 12-h light/12-h dark cycle, lights on at 6:00 am). Food and water were available ad libitum. Exposures to soman took place on P21; body weight at the time of soman exposure was 45–55 g. Experiments were conducted following the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council), and were approved by the Institutional Animal Care and Use Committees of the Uniformed Services University of the Health Sciences and the U.S. Army Medical Research Institute of Chemical Defense.

Soman exposure, SE, and drug treatments

Soman (pinacolyl methylphosphonofluoridate; obtained from the U.S. Army Edgewood Chemical Biological Center, Aberdeen Proving Ground, MD) was diluted in cold saline and administered to P21 rats via a single subcutaneous injection, at a dose that corresponds to $1.2 \times LD_{50}$, which is 74.4 μg/kg for the male rats[7] and, as we determined for the purposes of this study, 71.5 μg/kg for female P21 rats. To minimize the peripheral effects of soman, the rats were injected with 0.5-mg/kg atropine sulfate (intramuscularly; Vedco Inc., St. Joseph, MO) and 125-mg/kg 1-(2-hydroxyiminomethylpyridinium)-3-(4-carbamoylpyridinium)-2-oxa-propane dichloride (HI-6, intraperitoneally; Starks Associates, Buffalo, NY) within 1 min after soman injection. The soman-exposed rats were randomly divided into two treatment groups, which, at 60 min after soman exposure (about 50 min after the onset of SE), received either 3-mg/kg MDZ (Hospira Inc., Lake Forest, IL) or the combination of 10-mg/kg LY293558 (kindly provided by Raptor Pharmaceutical Corp., Novato, CA) and 50-mg/kg CRM (Sigma-Aldrich, St Louis, MO). The anticonvulsants were injected intramuscularly because this would be the administration route in convulsing humans. In deciding the appropriate anticonvulsant dose to use, we aim for the lowest dose that will completely stop seizure activity in less than 30 min, as high anticonvulsant doses may contribute to cardiorespiratory depression, particularly in an animal undergoing SE. The doses MDZ, LY293558, and CRM used in this study have been used previously in P21 rats exposed to $1.2 \times LD_{50}$ soman, from whom electroencephalography (EEG) recordings were obtained; both anticonvulsant treatments (administered 1 h postexposure) stopped the initial SE in less than 15 min.[28]

The occurrence of convulsive SE, in this study, was determined by behavioral observations based on the Racine scale,[29] as described previously.[19,27,28] Detailed analysis of seizure activity for 24 h after soman exposure has been done previously in EEG electrode–implanted P21 rats treated according to the same experimental protocol as in this study.[28] Electrode-implanted rats exposed to soman are euthanized after the completion of EEG recordings. We conduct neuropathology and behavioral studies in rats that have not been implanted with EEG electrodes, so as to avoid potential confounded variables (arising from the implantation of the electrodes or the stress associated with the presence of the headpiece and the electrodes, particularly during SE) which might influence the neuropathology and behavioral results.[30]

Fixation and tissue processing

At seven days, one, three, and six months after soman exposure, rats were deeply anesthetized with pentobarbital (75–100 mg/kg, intraperitoneally) and transcardially perfused with phosphate-buffered saline (PBS) (100 mL) followed by 4% paraformaldehyde (200 mL). The brains were removed and processed as we have described previously,[19] and subsequently stored at −20°C, until sectioning. A sliding microtome was used to cut a series of sections (1-in-5 series: for every five sections cut in series, one was kept) from the rostral extent of the amygdala to the caudal extent of the entorhinal cortex, at 40-µm thickness. One series of sections was mounted on slides (Superfrost Plus, Daigger, Vernon Hills, IL) in PBS for Nissl staining with cresyl violet. Adjacent series of sections were mounted on slides for Fluoro-Jade C (FJC) staining. All neuropathological analysis was done in a blind fashion.

FJC staining and analysis

The extent of neurodegeneration was assessed in the amygdala; piriform cortex; entorhinal cortex; a sample of neocortical area from the temporal lobe; and CA1, CA3, and hilar areas of the ventral hippocampus; we study neurodegeneration in the ventral hippocampus because we have seen previously that it displays significantly more severe neurodegeneration after soman exposure than the dorsal hippocampus.[31] The procedure used to identify irreversibly degenerating neurons by staining with FJC (Histo-Chem, Jefferson, AR) has been described in detail in previous reports.[15,19,27] Neurodegeneration was assessed by superimposing the FJC-stained sections onto tracings of the regions of interest from adjacent Nissl-stained sections, using the Stereo Investigator 9.0 (MicroBrightField, Williston, VT). The percentage of damaged tissue was estimated by considering the density of cells from Nissl-stained sections in comparison to the density of the FJC-stained cells, along the anterior to posterior extent, at 600-µm intervals. The rating scale used to score the extent of neuronal degeneration in each structure was as follows: 0 = no damage, 1 = minimal damage (1–10%), 2 = mild damage (11–25%), 3 = moderate damage (26–45%), and 4 = severe damage (>45%). Qualitative assessments were made from six sections per animal, and the average for each animal was recorded.

Estimation of neuronal loss

Design-based stereology was used to quantify the total number of neurons in Nissl-stained sections in the basolateral amygdala (BLA) and the CA1 hippocampal area, as described previously.[15,19,32] Sections were viewed with a Zeiss Axioplan 2ie fluorescent microscope (Oberkochen, Germany) equipped with a motorized stage and interfaced with a computer running Stereo Investigator 8.0 (MicroBrightField). Counting was done under a 63× oil immersion objective. The total number of Nissl-stained neurons was estimated using the optical fractionator probe, and, along with the coefficient of error (CE) – which was calculated by the software according to Gundersen (m = 1) and Schmitz-Hof (second estimation) equations[33,34] – was calculated by the Stereo Investigator 8.0.

To determine the number of Nissl-stained neurons in the BLA, one section in a series of five sections was analyzed (7 sections were used on average from each rat). The counting frame was 35 × 35 µm, the counting grid was 190 × 190 µm, and the dissector height was 12 µm. Nuclei were counted when the cell body came into focus within the dissector, which was placed 2 µm below the section surface. Section thickness was measured at every counting site, and the average mounted section thickness was 20 µm. An average of 321 neurons per rat was counted, and the average CE was 0.05 for both the Gundersen and Schmitz-Hof equations. For Nissl-stained neurons in the CA1 area, one section in a series of 10 sections was analyzed (7 sections on average). The counting frame was 20 × 20 µm, the counting grid was 250 × 250 µm, and the dissector height was 10 µm. Nuclei were counted when the cell body came into focus within the dissector which was placed 2 µm below the section surface. Section thickness was measured at every counting site, and the average mounted section thickness was 17.5 µm. An average of 222 neurons per rat was counted, and the CE was 0.05 for Gundersen (m = 1) and 0.055 for Schmitz-Hof (second estimation) equation.

Volumetric analysis

Nissl-stained sections containing the amygdala (sections were 200 µm apart) or the hippocampus (sections were 400 µm apart) were used to estimate stereologically the volume of these structures based on the previously described Cavalieri principle.[35] Sections were viewed with a Zeiss Axioplan 2ie fluorescent microscope (Oberkochen), equipped with a motorized stage, and interfaced with a computer running Stereo Investigator 9.0. The amygdala and the hippocampus were identified on slide-mounted sections under a 2.5× objective, based on the atlas of Paxinos and Watson,[36] and traced using Stereo Investigator 9.0; coordinates used for the amygdala were from bregma −2.6 to bregma −3.6, while coordinates used for the hippocampus were from bregma −2.3 to bregma −6.3. The volume was calculated using the stereological probe called Cavalieri estimator. An overlay of a rectangular lattice with a grid size of 300 µm was placed over the tracings of the amygdala and the hippocampus, and each point marked was counted to estimate the volume. For each animal, the CE was calculated to assure sufficient accuracy of the estimate (CE < 0.05).

Behavioral experiments

Anxiety-like behavior was assessed with the use of two tests, the open field and the acoustic startle response (ASR). The level of anxiety is affected by the estrous cycle, with the lowest anxiety observed during estrus.[37] At the time the rats were tested behaviorally (1, 3, and 6 months postexposure), the females were already cycling. To control for the phase of the estrous cycle, we tested all female rats during diestrus.

One day prior to testing, animals were acclimated to the open field apparatus (40 × 40 × 30 cm clear Plexiglas arena) for 20 min. On the test day, the rats were placed in the center of the open field, and activity was measured and recorded for 20 min, using an Accuscan Electronics infrared photocell system (Accuscan Instruments Inc., Columbus, OH).

Data were automatically collected and transmitted to a computer equipped with "Fusion" software (from Accuscan Electronics). Locomotion (distance traveled in centimeters), total movement time, and time spent in the center of the open field were analyzed. Anxiety-like behavior was measured as the ratio of the time spent in the center over the total movement time, expressed as a percentage of the total movement time.

ASR testing was conducted with the use of the Med Associates Acoustic Response Test System (Med Associates, Georgia, VT), which consists of weight-sensitive platforms inside individual sound-attenuating chambers. Each rat was individually placed in a ventilated holding cage. The holding cages are small enough to restrict extensive locomotion, but large enough to allow the subject to turn around and make other small movements. Each cage was placed on a weight-sensitive platform. Subjects' movements in response to stimuli were measured as a voltage change by a strain gauge inside each platform. The rats were acclimated to the apparatus in two sessions, on the two days preceding the test day. Startle stimuli consisted of 120-dB noise bursts (burst duration: 20 ms) presented 8 times. The interstimulus intervals ranged randomly from 15 to 25 s. An interfaced Pentium computer with Med Associates software recorded startle amplitude as the difference between the maximal voltage change during the startle period and the maximal voltage change during the no-stimulus periods, and assigned a value based on an arbitrary scale used by the software of the test system.

Detection of spontaneous recurrent seizures

Occurrence of spontaneous recurrent seizures (SRS) was evaluated during a 16-day period before the three- and six-month timepoints, by continuous video monitoring using a surveillance system. Each animal (treated with MDZ or LY293558 + CRM) was placed in a surveillance chamber, with water and food, for 96 h. SRS were identified based on the Racine scale.[28,29] Animals presenting generalized seizures stage 3, 4, and/or 5 at least 1 time within 96 h of monitoring were considered to have developed SRS.

Statistical analysis

Neurodegeneration scores were tested for statistically significant differences between the two groups for each structure separately, using the Mann–Whitney *U* test. Statistical values for neurodegeneration scores are presented as median and the interquartile range (IQR, the difference between the 75th and the 25th percentiles). Analysis of variance (ANOVA) followed by appropriate post hoc test (specified in the figure legends) was used to test for significant differences between three groups (soman-exposed group treated with MDZ, soman-exposed group treated with LY293558 + CRM, and control group not exposed to soman) in the stereological estimations of neuronal loss, volume of the amygdala and hippocampus, and in data obtained from the behavioral tests. ANOVA was also used to determine the effect of time (deterioration over time) on neuron loss and amygdala–hippocampal volume pathology. Statistical values for neuronal numbers, volume estimations, and behavioral results are presented as mean and standard error of the mean. The Fisher's exact test was used to test for significant differences between groups in the percentage of animals displaying SRS. For all tests, differences were considered significant when $P < 0.05$. Sample sizes (n) refer to the number of animals.

Results

Male and female rats were exposed to soman at P21. From 100 male rats, 90 developed SE (stage 3 and above), and 10 of them died before receiving treatment (before the 1 h post-exposure timepoint). From 90 female rats exposed to soman, 82 developed SE, and 7 of them died before receiving treatment. Rats who did not develop SE were not included in the neuropathology and behavior analyses. The occurrence of SE was determined by observations of behavioral seizures (see "Soman exposure, SE, and drug treatments" in the "Materials and methods" section). Brain damage and behavioral deficits were studied in the rats which developed SE.

Neuronal degeneration after treatment of soman-induced SE with MDZ – full protection by LY293558 + CRM treatment

The amount of neuronal degeneration was assessed at one week and one, three, and six months postexposure. At the seven-day timepoint, there were no degenerating neurons in any brain area of either male or female rats treated with MDZ or LY293558 + CRM. However, degenerating neurons were present at one, three, and six months postexposure, but only in the rats treated with MDZ. There were no significant differences in the neuronal degeneration scores between male and female rats treated with MDZ, except at three months postexposure when neurodegeneration was present in the CA1 and CA3 hippocampal areas of the male rats only (Figure 1). Thus, at one month postexposure, both males and females had moderate to severe neurodegeneration in the amygdala, piriform cortex, and CA3 hippocampal area; mild in the CA1 hippocampal area; minimal to mild in the hilus; and no neurodegeneration in the neocortical region sampled from the temporal lobe, or in the entorhinal cortex (Figure 1(C)). By three months postexposure, there was no neurodegeneration present except in the CA1 and CA3 hippocampal areas of the male rats (Figure 1(D)), where it was moderate to severe (CA1: median = 4, IQR = 2~4, $n = 8$; CA3: median = 3, IQR = 2~4, $n = 8$). At six months postexposure, again there was no neurodegeneration present except for a mild to moderate neurodegeneration in the CA1 and CA3 hippocampal areas of both male and female rats (Figure 1(E)).

Because there were no significant differences between male and female rats in their neurodegeneration scores, except for the hippocampus at three months postexposure, we grouped the data from males and females and compared the results from MDZ-treated rats with the results from the rats treated with LY293558 + CRM (Figure 2). Neurodegeneration was absent in the rats treated with LY293558 + CRM in all brain regions examined and at all three timepoints. In rats treated with MDZ, neurodegeneration was mild to severe in the amygdala (median = 3, IQR = 2.5~3), piriform cortex (median = 3, IQR = 2~4), and CA3 hippocampal area (median = 3, IQR = 3~3), while it was

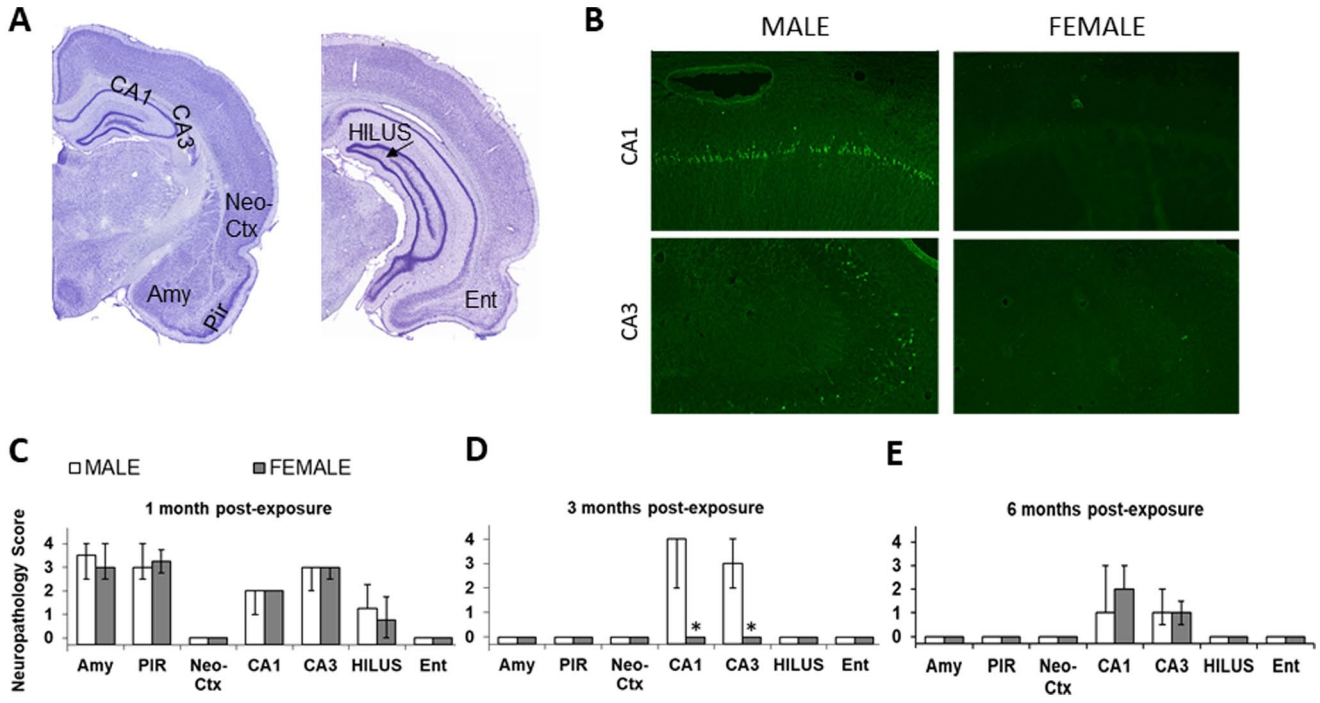

Figure 1. Time course of neuronal degeneration in male and female rats after exposure to soman at P21 and treatment with midazolam (MDZ) at 1 h postexposure. (A) Panoramic photomicrographs of Nissl-stained sections showing the brain regions evaluated by FJC staining. (B) Representative photomicrographs of FJC-stained sections from the CA1 and CA3 hippocampal areas of a male and a female rat, at three months postexposure; neurodegeneration was present only in the male rats. Total magnification is 100×. Scale bar is 50 μm. (C, D, E) Neuropathology scores (median and interquartile range; $n = 8$ for each group) in the amygdala (Amy), piriform cortex (Pir), neocortical region (Neo-Ctx), hippocampal areas (CA1, CA3, and HILUS), and entorhinal cortex (Ent), at one, three, and six months postexposure. *$P < 0.05$ (Mann–Whitney U test).
FJC: Fluoro-Jade C.

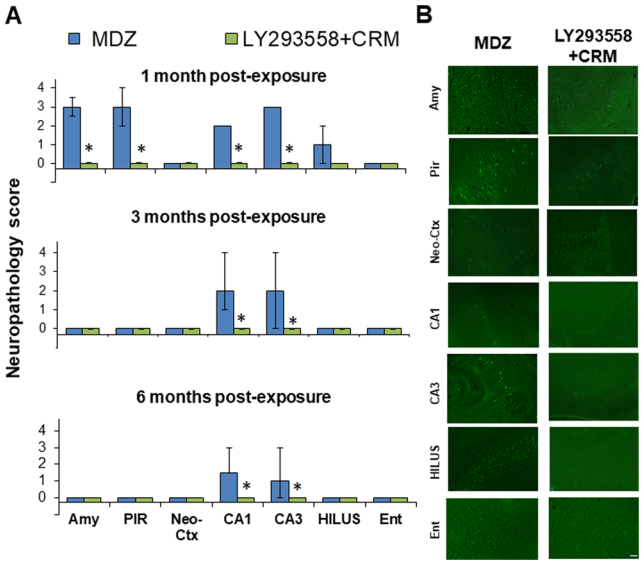

Figure 2. Complete protection against neuronal degeneration by LY293558 + CRM but not by MDZ. Male and female P21 rats were exposed to soman and treated with MDZ or LY293558 + CRM at 1 h postexposure; the data from males and females under each of the two anticonvulsant treatment groups have been combined. (A) Neuropathology scores (median and interquartile range) in the amygdala (Amy), piriform cortex (Pir), neocortical region (Neo-Ctx), hippocampal areas (CA1, CA3, and HILUS), and entorhinal cortex (Ent), at one, three, and six months postexposure. *$P < 0.05$ (Mann–Whitney U test, $n = 16$ for each group). (B) Representative photomicrographs of FJC-stained sections from the brain regions where neurodegeneration was studied. The sections are from animals evaluated at one month postexposure. Total magnification is 100×. Scale bar is 50 μm.
CRM: caramiphen; MDZ: midazolam; FJC: Fluoro-Jade C.

mild in the CA1 area (median = 2, IQR = 2~2) and absent to mild in the hilus (median = 1, IQR = 0~2), at one month postexposure. At three months postexposure, neurodegeneration in MDZ-treated rats was absent in all brain regions except for the CA1 and CA3 hippocampal areas, where it was minimal to moderate (median = 2, IQR = 1~4 for CA1; median = 2, IQR = 0~4 for CA3). By six months postexposure, there was still some neurodegeneration present in the CA1 (median = 1.5, IQR = 1.5~3) and CA3 (median = 1, IQR = 0~3) hippocampal areas of the MDZ-treated rats.

Loss of neurons in the BLA and the CA1 hippocampal area after treatment of soman-induced SE with MDZ – protection by LY293558 + CRM treatment

Neuronal loss in the BLA and the CA1 hippocampal area was evaluated at seven days and one, three, and six months after exposure to soman. There were no significant differences between male and female rats at any postexposure timepoint; the greater neuronal degeneration in the hippocampus of the MDZ-treated male rats at three months postexposure (in comparison with the females; Figure 1(B) and (C)) did not impact significantly the neuronal loss at three or six months postexposure. Thus, at three months postexposure, the total number of neurons in the CA1 hippocampal area was 478,098 ± 23,987 in the males ($n = 12$) and 482,764 ± 31,076 in the females ($n = 11$; $P = 0.09$), while in the BLA, it was 87,798 ± 1578 in the males and 88,124 ± 2178

in the females ($n = 11$–12; $P = 0.08$). At six months postexposure, the total number of neurons in the CA1 area was 348,128 ± 13,907 in the males ($n = 11$) and 401,554 ± 21,076 in the females ($n = 10$; $P = 0.065$), while in the BLA, it was 88,987 ± 2087 in the males and 89,589 ± 3879 in the females ($n = 10$–11 per group; $P = 0.12$). Thus, there was a tendency for greater neuronal loss in the males at six months postexposure, but the difference from the females was not statistically significant. For these reasons, the two sexes were grouped in the comparisons between MDZ-treated rats and LY293558 + CRM-treated rats.

In either the BLA or the CA1 area, there was no significant neuronal loss in the MDZ or the LY293558 + CRM group at seven days postexposure. However, in the BLA, there was significant reduction in the total number of neurons in the MDZ-treated rats at all three subsequent timepoints; at six months postexposure, there was also reduction of neurons in the rats treated with LY293558 + CRM (Figure 3). Specifically, at one month postexposure, the total number of neurons in the BLA of the MDZ group (97,990 ± 3974, $n = 24$) was lower than the total number of neurons in a control group of age-matched male and female rats that were not exposed to soman (117,166 ± 3888, $n = 24$; $P = 0.0053$); it was also lower than the total number of neurons in the LY293558 + CRM group (113,657 ± 4691, $n = 24$; $P = 0.0275$), which did not differ from the controls ($P = 0.8255$). At three months postexposure, the total number of neurons in the MDZ group (87,960 ± 6888, $n = 23$) was lower than the total number of neurons in a control group (127,849 ± 7974, $n = 24$; $P = 0.004$) and the total number of neurons in the LY293558 + CRM group (118,260 ± 5691, $n = 24$; $P = 0.0081$), which again did not differ from the controls ($P = 0.5874$). At six months postexposure, the total number of neurons in the MDZ group (79,765 ± 4691, $n = 20$) was lower than the total number of neurons in the control group (130,978 ± 6980, $n = 20$; $P = 0.0001$) and the total number of neurons in the LY293558 + CRM group (108,981 ± 5640, $n = 22$; $P = 0.0021$), which was also lower compared with the controls ($P = 0.0253$; Figure 3(C)). There was also an overall effect of time (deterioration over time) for the MDZ-treated animals, with significant decrease of neuronal count from seven days to six months ($P = 0.02$), but there was no significant difference in the neuronal count with the progression of time from one month to six months postexposure ($P = 0.0647$).

In the CA1 hippocampal area, there was a significant reduction in the total number of neurons in the MDZ-treated rats at three and six months postexposure (Figure 4). Thus, at one month postexposure, the total number of neurons in the MDZ group (603,431 ± 29,466, $n = 23$) did not differ significantly from the total number of neurons in the control group (680075 ± 31,776, $n = 24$) or the total number of neurons in the LY293558 + CRM group (635,870 ± 30,681, $n = 24$; $P = 0.2169$). However, at three months postexposure, the total number of neurons in the MDZ group (480,431 ± 27,531, $n = 23$) was lower than the total number of neurons in the control group (684,375 ± 33,765; $n = 24$; $P = 0.025$) and the total number of neurons in the LY293558 + CRM group (633,047 ± 35658, $n = 24$; $P = 0.044$), which did not differ from the controls ($P = 0.7053$). At six months postexposure, the total number of neurons in the MDZ group (416,874 ± 38,970, $n = 23$) was lower than the total number of neurons in the control group (690,175 ± 40,870, $n = 24$; $P = 0.003$) and the total number of neurons in the LY293558 + CRM group (616,338 ± 35,791, $n = 24$; $P = 0.015$), which again was not significantly different from the controls ($P = 0.566$; Figure 4(C)). There was also an overall effect of time (deterioration over time) for the MDZ-treated animals, with significant reduction in neuronal count from one to six months ($P = 0.04$), without significant difference in the neuronal count between the three- and six-month timepoint ($P = 0.065$).

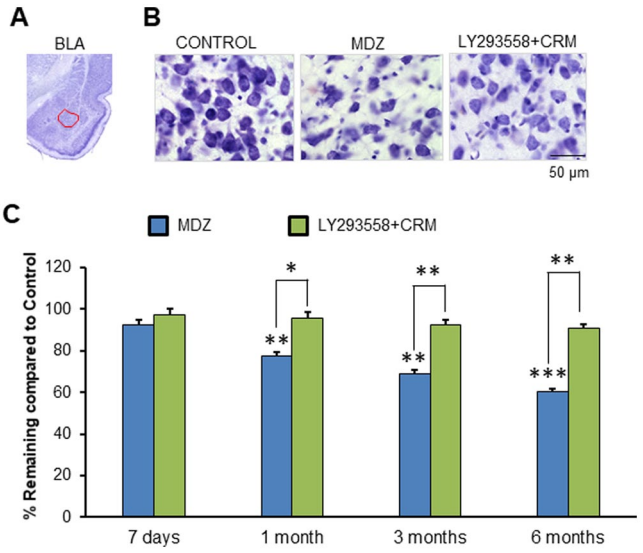

Figure 3. Protection against neuronal loss in the basolateral amygdala (BLA) by LY293558 + CRM but not by MDZ. Male and female P21 rats were exposed to soman and treated with MDZ or LY293558 + CRM, 1 h after exposure; neuronal loss in the BLA was assessed at seven days and one, three, and six months postexposure (data from male and female rats are combined). (A) Panoramic photomicrograph of a Nissl-stained section showing the BLA, where neuronal loss was assessed. (B) Photomicrographs of Nissl-stained sections from the BLA of a representative animal from the control group (not exposed to soman), the MDZ-treated group, and the LY293558 + CRM-treated group, taken at one month postexposure. Total magnification is 630×. (C) Group data of stereological estimation of the total number of neurons in the BLA as percent of the control group. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ for comparisons between the control, the MDZ, and the LY293558 + CRM groups (ANOVA, LSD post hoc test). CRM: caramiphen; MDZ: midazolam; ANOVA: analysis of variance; LSD: Least Significant Difference.

Long-term reduction in amygdala and hippocampal volume after soman-induced SE and treatment with MDZ – full protection by LY293558 + CRM treatment

Amygdala and hippocampal volumes were examined at one, three, and six months postexposure; there were no significant differences between male and female rats at any postexposure timepoint and, therefore, the data from the two sexes were grouped. The volume of the amygdala was found reduced at all three postexposure timepoints, but only in the group treated with MDZ (Figure 5). Thus, one month after soman exposure, amygdala volume in the MDZ group (9.85 ± 0.6 mm^3, $n = 24$) was significantly smaller compared with either the control group (12.48 ± 0.4 mm^3, $n = 24$; $P = 0.0011$) – which consisted of age-matched male and female rats that were not exposed to soman – or the LY293558 + CRM group (11.81 ± 0.4 mm^3, $n = 24$; $P = 0.0133$), which was not significantly different from the controls ($P = 0.582$). At three months postexposure, the volume of the amygdala was reduced further in the MDZ group

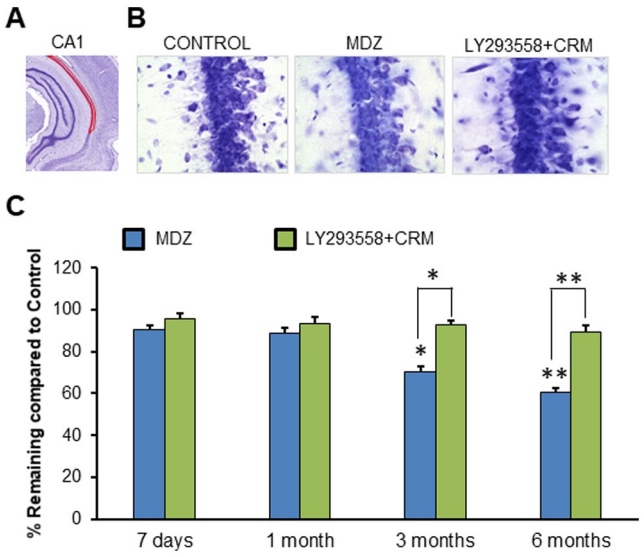

Figure 4. Complete protection against neuronal loss in the CA1 hippocampal area by LY293558 + CRM but not by MDZ. Male and female P21 rats were exposed to soman and treated with MDZ or LY293558 + CRM, 1 h after exposure; neuronal loss in the CA1 hippocampal area was assessed at seven days and one, three, and six months later (data from males and females are combined). (A) Panoramic photomicrograph of a Nissl-stained section showing the hippocampal CA1 area where neuronal loss was assessed. (B) Representative photomicrographs of Nissl-stained sections from the CA1 hippocampal area of a representative animal from the control group (not exposed to soman), the MDZ-treated group, and the LY293558 + CRM-treated group, taken at six months postexposure. Total magnification is 630×. (C) Group data of stereological estimation of total number of neurons in the CA1 hippocampal area as percent of the control group. $^{*}P < 0.05$, $^{**}P < 0.01$ for comparisons between the control, the MDZ, and the LY293558 + CRM groups (ANOVA, LSD post hoc test).
CRM: caramiphen; MDZ: midazolam; ANOVA: analysis of variance; LSD: least significant difference.

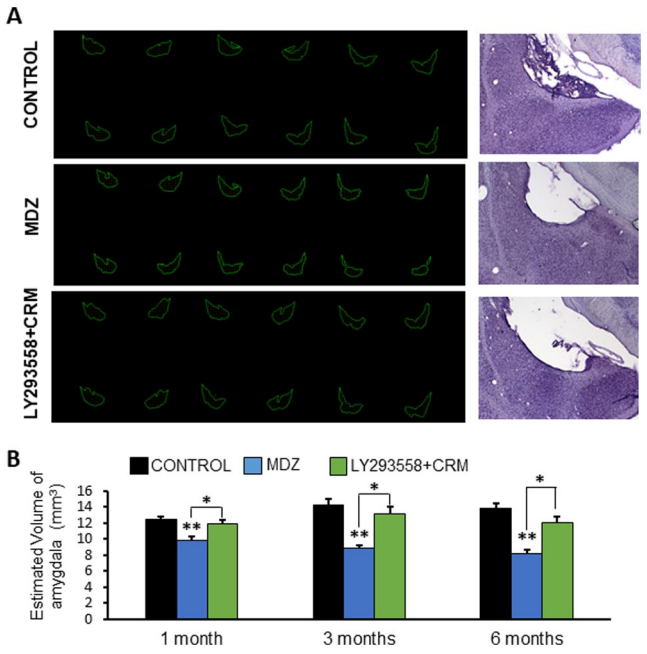

Figure 5. Complete protection against amygdala atrophy by LY293558 + CRM but not by MDZ. Male and female P21 rats were exposed to soman and treated with MDZ or LY293558 + CRM, 1 h after exposure; the volume of the amygdala was examined at one, three, and six months postexposure (data from male and female rats are combined). (A) Tracings of the amygdala in series of slices (left) and representative photomicrographs (right) from a control rat (not exposed to soman), a soman-exposed rat treated with MDZ, and a soman-exposed rat treated with LY293558 + CRM. (B) Group data showing the estimated volume of the amygdala for the control group (not exposed to soman), the MDZ group, and the LY293558 + CRM group ($n = 24$ for each of the 3 groups). $^{*}P < 0.05$, $^{**}P < 0.01$ (ANOVA with Bonferroni post hoc test). Amygdala volume at six months was significantly smaller than that at one month ($P = 0.04$; Tuckey HSD post hoc test).
CRM: caramiphen; MDZ: midazolam; ANOVA: analysis of variance; HSD: honest Significant Difference.

($9.0 \pm 0.5\,mm^3$, $n = 24$) and was significantly smaller compared with either the control group ($14.2 \pm 1.3\,mm^3$, $n = 24$; $P = 0.007$) or the LY293558 + CRM group ($13.0 \pm 1.2\,mm^3$, $n = 24$; $P = 0.0108$), which was not significantly different from the controls ($P = 0.645$). At six months postexposure, the volume of the amygdala in the MDZ group ($8.18 \pm 0.9\,mm^3$, $n = 24$) was again significantly smaller than either the control group ($13.8 \pm 1\,mm^3$, $n = 24$; $P = 0.004$) or the LY293558 + CRM group ($12.05 \pm 1.1\,mm^3$, $n = 24$; $P = 0.0188$), which did not differ significantly from the controls ($P = 0.438$; Figure 5(B)). There was a deterioration of amygdala atrophy over time, as amygdala volume at six months was significantly smaller than that at one month ($P = 0.04$).

Hippocampal volume was reduced in the MDZ-treated group, at three and six months postexposure (Figure 6). Thus, one month after soman exposure, the volume of the hippocampus in the MDZ group ($60.4 \pm 5\,mm^3$, $n = 24$) or the LY293558 + CRM group ($65.2 \pm 2.9\,mm^3$, $n = 24$) did not differ significantly from the hippocampal volume in the control group ($65.8 \pm 3\,mm^3$, $n = 24$; $P = 0.541$). At three months postexposure, the volume of the hippocampus in the MDZ group ($51.1 \pm 4\,mm^3$, $n = 24$) was significantly smaller compared with either the control group ($68.4 \pm 3\,mm^3$, $n = 24$; $P = 0.015$) or the LY293558 + CRM group ($63.1 \pm 3\,mm^3$, $n = 24$; $P = 0.0369$), which was not significantly different from the controls ($P = 0.509$; Figure 6). At six months postexposure, hippocampal volume in the MDZ group ($48.2 \pm 5\,mm^3$, $n = 24$) was again significantly smaller than either the control group ($70.2 \pm 2\,mm^3$, $n = 24$; $P = 0.002$) or the LY293558 + CRM group ($62.2 \pm 3.1\,mm^3$, $n = 24$; $P = 0.0241$), which did not differ significantly from the controls ($P = 0.2803$; Figure 6(B)). There was a deterioration of hippocampal atrophy over time, as hippocampal volume was significantly smaller at three months ($P = 0.046$) and six months ($P = 0.030$) compared with the one-month timepoint.

Long-term increases in anxiety-like behavior after soman-induced SE and treatment with MDZ – full protection by LY293558 + CRM treatment

To determine whether the long-term neuropathology had produced behavioral deficits, we used the open field and the ASR tests to measure the level of anxiety, at one, three, and six months after soman exposure. There were no significant differences between male and female rats in anxiety-like behavior, in any of the three groups (control, MDZ-treated, and LY293558 + CRM-treated) at any postexposure timepoint (female rats were tested during diestrus; see the "Materials and methods" section). However, because differences between sexes in anxiety-like behavior are of interest,[38] even when absent, we did not group the data from the two sexes.

In the open field, at one month postexposure, the time spent in the center of the field by male rats treated with MDZ ($6.3 \pm 0.9\%$ of the total movement time, $n = 12$) was significantly less compared with the age-matched

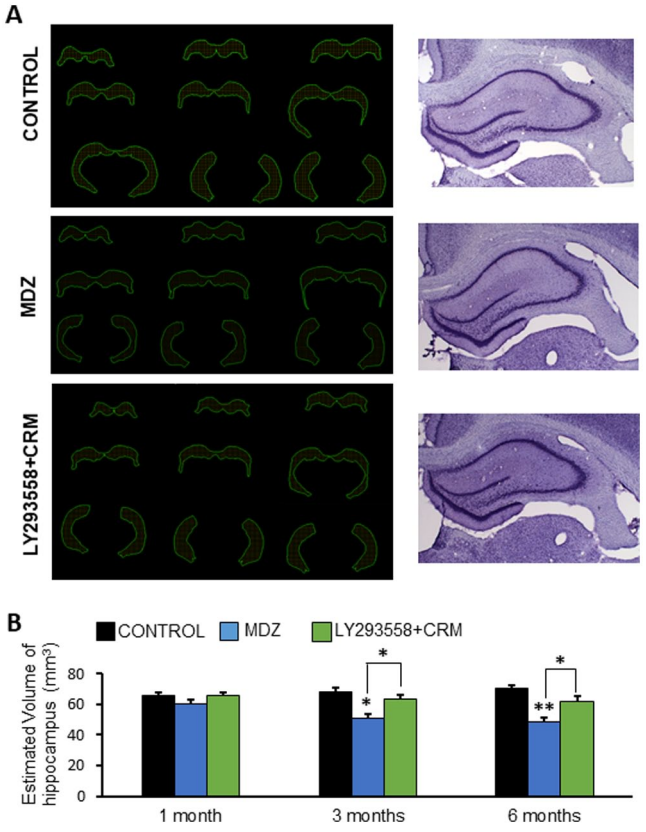

Figure 6. Complete protection against hippocampal atrophy by LY293558 + CRM but not by MDZ. Male and female P21 rats were exposed to soman and treated with MDZ or LY293558 + CRM, 1 h after exposure; the volume of the hippocampus was examined at one, three, and six months later (data from males and females are combined). (A) Tracings of the hippocampus in series of slices (left) and representative photomicrographs (right) from a control rat (not exposed to soman), a soman-exposed rat treated with MDZ, and a soman-exposed rat treated with LY293558 + CRM. (B) Group data showing the estimated volume of the hippocampus for the control group (not exposed to soman), the MDZ group, and the LY293558 + CRM group ($n = 24$ for each of the 3 groups). $^{*}P < 0.05$, $^{**}P < 0.01$ (ANOVA with Bonferroni post hoc test). Hippocampal volume was significantly smaller at three and six months compared with the one-month timepoint ($P = 0.046$ and $P = 0.030$ for the three- and six-month timepoints, respectively; ANOVA, Tuckey HSD post hoc test). CRM: caramiphen; MDZ: midazolam; ANOVA: analysis of variance; HSD: honest significant difference.

control group (13.9 ± 1% of the total movement time, $n = 12$; $P = 0.00009$) or the LY293558 + CRM group (12.1 ± 1.1% of the total movement time, $n = 12$; $P = 0.0007$), which did not differ from the control ($P = 0.4226$; Figure 7(A), left panel). The distances traveled by the control group (2520 ± 252 cm), the MDZ group (2651 ± 235 cm), and the LY293558 + CRM group (2457 ± 212 cm) were not significantly different ($P = 0.8265$). In MDZ-treated female rats, the time spent in the center of the open field (7.5 ± 0.8% of the total movement time, $n = 10$) at one month postexposure was also significantly less compared with the age-matched control group (15.2 ± 1.1% of the total movement time, $n = 10$; $P = 0.00008$) or the LY293558 + CRM group (13.6 ± 1.1% of the total movement time, $n = 10$; $P = 0.0006$), which did not differ from the control ($P = 0.5102$; Figure 7(A), left panel). The distances traveled by the female control group (2089 ± 190 cm), the MDZ group (2176 ± 210 cm), and the LY293558 + CRM group (1991 ± 250 cm) were not significantly different ($P = 0.8362$).

Three months after exposure to soman, the time spent in the center of the open field by male rats treated with MDZ (10.9 ± 0.9% of the total movement time, $n = 12$) was significantly less compared with the age-matched control group (15.9 ± 1.2% of the total movement time, $n = 10$; $P = 0.033$) or the LY293558 + CRM group (14.7 ± 0.7% of the total movement time, $n = 12$; $P = 0.0198$), which did not differ from the control ($P = 0.711$; Figure 7(A), middle panel). The distances traveled by the control group (2350 ± 330 cm), the MDZ group (2580 ± 290 cm), and the LY293558 + CRM group (2189 ± 330 cm) were not significantly different ($P = 0.6495$). In MDZ-treated female rats, the time spent in the center of the open field (11.2 ± 0.9% of the total movement time, $n = 10$) was also significantly less compared with the age-matched control group (16.9 ± 1% of the total movement time, $n = 10$; $P = 0.003$) or the LY293558 + CRM group (16.2 ± 2.1% of the total movement time, $n = 10$; $P = 0.0042$), which did not differ from the control ($P = 0.2841$; Figure 7(A), middle panel). The distances traveled by the female control group (2192 ± 210 cm), the MDZ group (2281 ± 280 cm), and the LY293558 + CRM group (2189 ± 250 cm) were not significantly different ($P = 0.95$).

Six months postexposure, the time spent in the center of the open field by male rats treated with MDZ (9.8 ± 0.8% of the total movement time, $n = 9$) was significantly less compared with the age-matched control group (18.1 ± 1% of the total movement time, $n = 10$; $P = 0.00001$) or the LY293558 + CRM group (16.5 ± 1% of the total movement time, $n = 10$; $P = 0.0001$), which did not differ from the control ($P = 0.4541$; Figure 7(A), right panel). The distances traveled by the control group (2397 ± 290 cm), the MDZ group (2568 ± 300 cm), and the LY293558 + CRM group (2299 ± 300 cm) were not significantly different ($P = 0.7694$). In MDZ-treated female rats, the time spent in the center of the open field (9.5 ± 0.8% of the total movement time, $n = 9$) was significantly less compared with the age-matched control group (17.1 ± 1% of the total movement time, $n = 10$; $P = 0.0002$) or the LY293558 + CRM group (16.2 ± 1.4% of the total movement time, $n = 10$; $P = 0.0008$), which did not differ from the control ($P = 0.8317$; Figure 7(A), right panel). The distances traveled by the female control group (2078 ± 212 cm), the MDZ group (2398 ± 287 cm), and the LY293558 + CRM group (2005 ± 185 cm) were not significantly different ($P = 0.4549$).

In the ASR test, at one month postexposure, the startle amplitude of male rats treated with MDZ (17.8 ± 0.6, $n = 12$) was significantly higher compared with the control group (13.8 ± 1, $n = 10$; $P = 0.0094$) or the LY293558 + CRM group (12.8 ± 1, $n = 12$; $P = 0.007$), which did not differ from the control ($P = 0.7106$; Figure 7(B), left panel). The startle amplitude of female rats treated with MDZ (18.9 ± 1.3, $n = 10$) was also significantly higher compared with the control group (12.2 ± 0.9, $n = 10$; $P = 0.0009$) or the LY293558 + CRM group (10.3 ± 1.2, $n = 10$; $P = 0.00001$), which did not differ from the control ($P = 0.4794$; Figure 7(B), left panel). Three months after exposure to soman, the startle amplitude of male rats treated with MDZ (18.5 ± 2, $n = 12$) was significantly higher compared with the control group (11.9 ± 1.1, $n = 10$; $P = 0.0112$) or the LY293558 + CRM group (13.4 ± 1, $n = 12$; $P = 0.0451$), which did not differ from the control ($P = 0.7633$; Figure 7(B), middle panel). The startle amplitude of female rats treated with MDZ (17.9 ± 1.5, $n = 10$) was also significantly higher compared

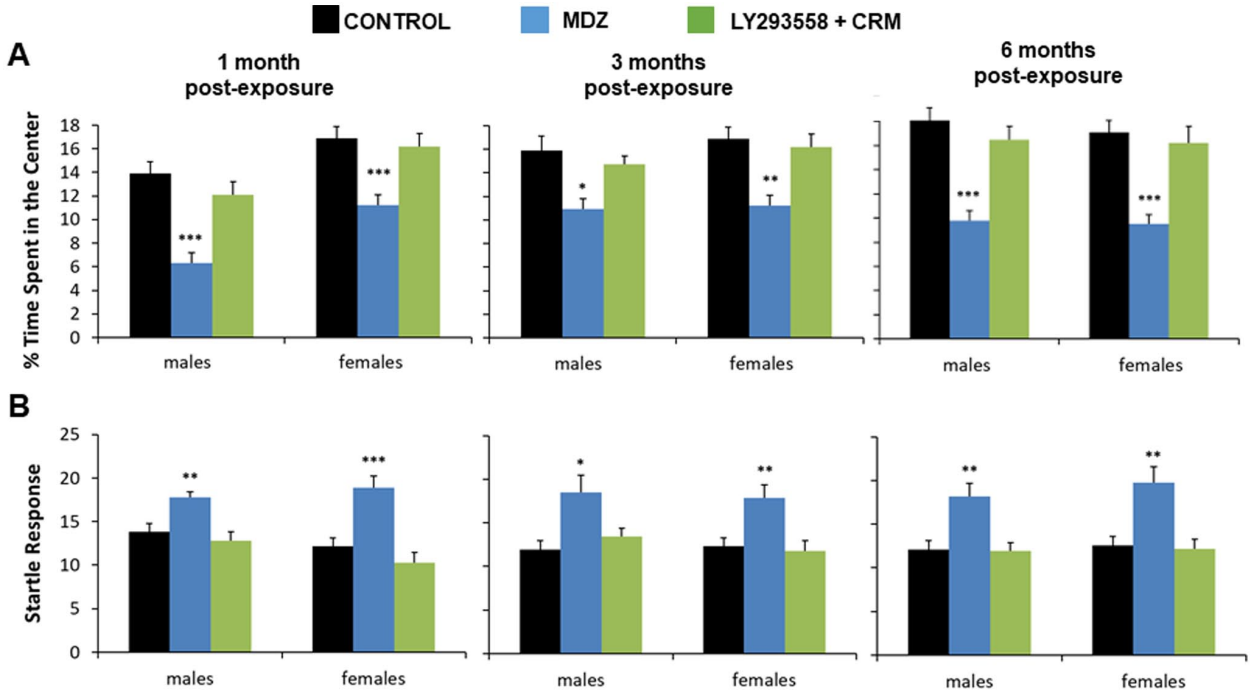

Figure 7. Complete protection against increased anxiety by LY293558 + CRM but not by MDZ. Male and female P21 rats were exposed to soman and treated with MDZ or LY293558 + CRM, 1 h after exposure; anxiety-like behavior was tested at one, three, and six months after soman exposure (female rats were tested during diestrus). (A) Group data of the percent of the total movement time that was spent in the center of the open field by the control rats (not exposed to soman), the MDZ-treated rats, and the LY293558 + CRM-treated rats. (B) Group data of the amplitude of the startle response to 120-dB acoustic stimulus bursts for the control groups (not exposed to soman) and the two experimental groups. Sample size *n* ranges from 10 to 12 rats in the control groups, 11 to 12 rats in the MDZ groups, and 11 to 12 rats in the LY293558 + CRM groups. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ (one-way ANOVA with Dunnett's test post hoc).
CRM: caramiphen; MDZ: midazolam; ANOVA: analysis of variance.

Table 1. Percentages of male and female rats displaying spontaneous recurrent seizures after soman exposure and treatment with midazolam (MDZ) or LY293558 + caramiphen (CRM).

	MDZ			LY293558 + CRM		
	Males	Females	Males + Females	Males	Females	Males + Females
3 months after soman exposure	40% (8/20)	0%† (0/20)	20% (8/40)	0%** (0/20)	0% (0/20)	0%** (0/40)
6 months after soman exposure	50% (6/12)	30% (3/12)	37.5% (9/24)	0%* (0/12)	0% (0/12)	0%** (0/24)

†$P < 0.01$ in comparison with the males in the MDZ group. *$P < 0.05$ and **$P < 0.01$ in comparison with the respective MDZ groups (Fisher's exact test).

with the control group (12.3 ± 0.95, $n = 10$; $P = 0.0095$) or the LY293558 + CRM group (11.8 ± 1.2, $n = 10$; $P = 0.0047$), which did not differ from the control ($P = 0.9561$; Figure 7(B), middle panel). Six months after exposure to soman, the startle amplitude of male rats treated with MDZ (18.2 ± 1.5, $n = 10$) was significantly higher compared with the control group (12.1 ± 1, $n = 10$; $P = 0.0031$) or the LY293558 + CRM group (11.9 ± 1, $n = 10$; $P = 0.0023$), which did not differ from the control ($P = 0.9917$; Figure 7(B), right panel). The startle amplitude of female rats treated with MDZ (19.8 ± 1.8, $n = 9$) was also significantly higher compared with the control group (12.6 ± 1, $n = 10$; $P = 0.0018$) or the LY293558 + CRM group (12.2 ± 1.1, $n = 10$; $P = 0.0011$), which did not differ from the control ($P = 0.9715$; Figure 7(B), right panel).

Development of SRS

Soman-exposed rats treated with MDZ or LY293558 + CRM were monitored for appearance of SRS for 16 days before the three-month timepoint and 16 days before the six-month timepoint. None of the rats treated with LY293558 + CRM presented SRS; the results are presented in Table 1. The difference of LY293558 + CRM-treated rats from MDZ-treated rats was statistically significant when the males of the two treatment groups were compared at three months ($P = 0.0035$) or six months ($P = 0.0137$) postexposure, and when the grouped data (males and females) from the two treatment groups were compared at three months ($P = 0.0013$) and six months ($P = 0.0071$) postexposure (Table 1). In the MDZ-treated group, the difference between males and females was significant at three months ($P = 0.0033$) but not at six months ($P = 0.4003$) postexposure.

Discussion

In this study, we compared the neuropathological outcome and its time course when SE induced by exposure of male and female P21 rats to soman is treated with MDZ or LY293558 + CRM at 1 h after soman injection. We found that only rats treated with MDZ presented evidence of substantial

brain damage. Neuronal degeneration was absent in rats treated with LY293558 + CRM, while in MDZ-treated rats, there was significant neurodegeneration in the amygdala, hippocampus, and piriform cortex, one month after soman exposure; degenerating neurons were still present in the hippocampus at the three- and six-month timepoints. Significant neuronal loss was found in the BLA of the MDZ-treated group, at one, three, and six months postexposure, while the LY293558 + CRM group also had some neuronal loss at the six-month timepoint. In the CA1 hippocampal area, there was no neuronal loss in the MDZ-treated group at one month, but significant neuronal loss was found at three and six months postexposure; in the LY293558 + CRM group, there was no significant neuronal loss in the CA1 area at any timepoint. Both the amygdala and the hippocampus were reduced in volume, only in the MDZ-treated group; while hippocampal atrophy was present at three and six months postexposure, amygdala atrophy was already present at one month and deteriorated subsequently. Anxiety-like behavior was increased at one, three, and six months postexposure, only in the MDZ-treated group. SRS also appeared only in the MDZ-treated rats, at three and six months postexposure. No significant differences were found between male and female rats, except that at the three-month timepoint, there were no degenerating neurons and no SRS in the female rats of the MDZ-treated group.

The primary cause of brain damage by acute nerve exposure appears to be the intense and prolonged SE,[39–41] although other mechanisms may also participate.[42] It has become clear from studies in both animals and humans that prolonged SE can cause neuronal death via excitotoxic mechanisms.[43,44] Seizure activity is associated with excessive release of the neurotransmitter glutamate, which acts on AMPA, kainate, and NMDA receptors to further reinforce seizures by neuronal depolarization, and to damage neurons by causing high and sustained elevations of intracellular Ca^{++} in both neurons and glia cells.[44–46] MDZ counteracts neuronal depolarization and hyperexcitability by enhancing the responsiveness of $GABA_A$ receptors to GABA, the major inhibitory neurotransmitter in the brain. However, postsynaptic $GABA_A$ receptors are internalized and downregulated as SE progresses,[47–49] which deprives neurons from the targets of MDZ, thus reducing its efficacy. This, along with the relatively rapid clearance rate of MDZ, may explain the reoccurrence of prolonged SE after a prompt but transient cessation by MDZ administration[28] and the resulting neuropathology. In contrast to the downregulation of $GABA_A$ receptors, AMPA, GluK1, and NMDA receptors are all upregulated by prolonged seizure activity.[50–52] NMDA receptors and subtypes of AMPA and kainate receptors allow the influx of calcium into cells,[53–55] leading to Ca^{++} overload during prolonged seizure activity. Blocking AMPA and GluK1 receptors with LY293558, in synergism with blockade of NMDA receptors by CRM, reduces significantly the total duration of SE,[28] and, as this study shows, it can also provide full protection against neuronal damage, even when the treatment is delayed to 1 h after soman exposure.

In the group treated with MDZ, neurodegeneration was absent at seven days postexposure, but was significant at subsequent timepoints, particularly at one month after soman exposure. Neurodegeneration at one month was mild in the CA1 hippocampal area and moderate to severe in the amygdala, which may have contributed to the earlier loss of neurons in the BLA and atrophy of the amygdala (evident at 1 month postexposure) compared with the hippocampus. On the other hand, at three and six months postexposure, degenerating neurons were still present in the hippocampus but not in the amygdala, suggesting that there could be further neuronal loss in the hippocampus at a later timepoint beyond the six months. The time course data from both neuronal loss and atrophy of the amygdala and the hippocampus show a deterioration of brain damage over time. Even in the LY293558 + CRM group, which exhibited no brain damage in nearly all measures, significant neuronal loss was found in the BLA, but only at six months postexposure (apparently, some neurodegeneration had occurred in the amygdala of the LY293558 + CRM group, but it was not present at the postexposure timepoints that we examined). Also, female rats from the MDZ-treated group did not display SRS until six months postexposure. We are not aware of any previous studies revealing the progressively deteriorating neuropathology after nerve agent exposure; however, long-lasting brain damage has been seen in animals exposed to nerve agents as well as in human victims of the sarin attacks in Matsumoto in 1994 and Tokyo in 1995[56]; in addition, progressively deteriorating pathology after prolonged SE of different etiologies has been previously found in animals[57] and humans.[58]

The neuropathology found in the MDZ-treated group was accompanied by the appearance of SRS in a subsection of the rats and an increase in anxiety-like behavior. These neurological and behavioral manifestations – which are similar to the most commonly found long-term morbidities in human victims of nerve agent exposure[56] – are probably a result of the neuronal damage and loss, particularly in the amygdala and the hippocampus, two brain regions that play a central role in both seizure generation[59,60] and anxiety.[61–63] Although we did not find sex-related differences in anxiety-like behavior, the males appeared to be more vulnerable to epileptogenesis, as SRS appeared earlier and in a somewhat greater percentage of the male rats. The absence of SRS in the female rats at three months postexposure coincided with an absence of degenerating neurons in the hippocampus; the latter suggests a difference between male and female rats in the temporal pattern of neuronal degeneration in the hippocampus, but its long-term neurological significance may not be substantial, since at six months postexposure, the difference between the two sexes in the percentage of animals displaying SRS was not statistically significant.

A major concern for survivors of acute nerve agent poisoning is the potential for long-lasting or permanent brain damage caused primarily by SE that is not treated promptly. The results of this study suggest that brain damage may indeed be long-lasting or permanent if nerve agent–induced SE is treated with MDZ and with a relative delay after SE onset, while antiglutamatergic anticonvulsant treatment consisting of tezampanel and CRM can provide full neuroprotection. The young age of the rats at the time of soman exposure (P21 would roughly correspond to a 3- to 5-year-old child if we take into account the developmental stage of the brain)[64]

did not appear to be a factor for a favorable long-term outcome when SE was treated with MDZ, and if we attempt to approximate the six-month postexposure period to human years, it would roughly correspond to 17 years.[65] Therefore, when immediate treatment of nerve agent–induced SE is not feasible, administering MDZ at a delayed timepoint will require follow-up treatments for the control of reoccurring SE, in short term, and additional long-term neuroprotective treatments – that may or may not be successful – aimed at halting the processes that cause deteriorating neuropathology. In contrast, an effective antiglutamatergic initial treatment may prevent damage even when offered with a delay, without the need of additional interventions.

AUTHORS' NOTE

The views expressed are those of the authors and do not reflect the official policy or position of the Uniformed Services University of the Health Sciences, the Department of the Defense, or the United States government.

AUTHORS' CONTRIBUTIONS

All authors have reviewed and agreed to the publication of the article. MFMB and VA-A initiated the research and designed the study. THF, JPA, and KR conducted experiments. THF, VA-A, and MFMB analyzed and interpreted the data. VA-A wrote the article. THF, JPA, and MFMB contributed to the writing of the article.

DECLARATION OF CONFLICTING INTERESTS

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