

# Effects of Sarin on Temperature and Activity of Rats as a Model for Gulf War Syndrome Neuroregulatory Functions

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Coexposure to subclinical levels of nerve gas and to heat stress may have induced some of the clinical symptoms of the Gulf War Syndrome. We tested the hypothesis that single or repeated subclinical exposure to sarin, particularly under conditions of heat stress, would impair regulation of body temperature and locomotor activity. Male F344 rats were housed at 25°C or under mild heat stress at 32°C and were exposed 1 h/day for 1, 5, or 10 days to 0, 0.2, or 0.4 mg/m<sup>3</sup> of sarin in a nose-only exposure system. Body temperature and activity were monitored continuously by telemetry during exposure and 1 month postexposure. Exposed rats showed no clinical symptoms of toxicity such as tremors, despite evidence of reduced red blood cell cholinesterase activity. Heat stress consistently elevated body temperature in unexposed animals, particularly during the dark period when animals are most active. Inhalation of sarin gas at the two subclinical levels did not affect body temperature acutely in a biologically meaningful manner after the first exposure nor after 5 or 10 repeated exposures, either at thermoneutral ambient temperature or during chronic heat stress. There were no consistent effects of sarin or housing temperature on activity. The data suggest that subclinical levels of sarin have minimal effects on temperature regulation and locomotor activity under these observation conditions. © 2002 Elsevier

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**Key Words:** subclinical exposure; telemetry; anticholinesterase; heat stress.

Following active duty in the Persian Gulf between August 1990 and June 1991, some U.S. military personnel manifested symptoms designated as the Gulf War Syndrome. Expression of the syndrome differs among individuals but can include arthralgia, weakness, fatigue, headache, memory loss, and increased susceptibility to infections (IOM, 1995). Etiology of the syndrome is currently unknown, but it has been hypothesized that exposure to subclinical levels of nerve gas might

cause subtle neuroimmune abnormalities that could manifest in the clinical symptoms of the syndrome (Haley *et al.*, 1999). Similar clinical symptoms were noted in individuals living in the area surrounding Matsumoto City, Japan, following the acute sarin poisoning that occurred inside the city on June 27, 1994 (Nakajima *et al.*, 1999). However, very little experimental evidence is available on the physiological effects of single or repeated exposures to low levels of nerve gas.

One mechanism by which organophosphate agents such as sarin gas exert their physiological effects is by irreversibly inhibiting the enzyme acetylcholinesterase (AChE), resulting in central accumulation of acetylcholine and overexcitation of cholinergic neurons. High levels of exposure result in seizures, which increase the release of glutamate and cause toxicity to surrounding cells (Solberg and Belkin, 1997). The control of body temperature is integrated in the central nervous system via cholinergic pathways, and acute exposure to organophosphates can cause profound changes in body temperature lasting several days (Gordon, 1996). A smaller accumulation of acetylcholine because of a low-level exposure might also alter the capability for normal thermoregulation, but this hypothesis is currently untested. In addition to the inhibition of cholinesterases, a range of noncholinesterase effects of organophosphates has been observed (Ray, 1998). As an example pertinent to this investigation, hippocampal slices exposed to low levels of sarin showed altered release of  $\gamma$ -amino butyric acid and glutamate neurotransmitters involved in thermoregulation (Rocha *et al.*, 1998).

Fatigue is a major symptom of the Gulf War Syndrome and also has been documented in female greenhouse workers exposed to organophosphorous pesticides (Bazylewicz-Walczak *et al.*, 1999). Central fatigue resulting in lethargy is postulated to occur when neurotransmitter activity in the central nervous system is altered (Newsholme *et al.*, 1992; Bailey *et al.*, 1993), and the hypothesis has been put forward that alterations in GABAergic and cholinergic transmission are involved in fatigue syndromes (Corrigan *et al.*, 1994). Both sarin and soman have decreased locomotor activity in rats when injected ip in doses large enough to inhibit AChE activity in the blood

(Nieminen *et al.*, 1990). The effect of single or repeated exposure to inhalation of subclinical doses on activity levels is not known.

Another feature of the Gulf War is the possibility that subclinical exposures to nerve gas might have occurred under conditions of heat stress. It is known that ambient heat and cold stress modulate an animal's sensitivity to higher levels of anticholinesterase agents (Gordon, 1996). Thus, it is possible that the combination of subclinical exposure to nerve gas and heat stress may have resulted in clinical symptoms of the Gulf War Syndrome. We tested the hypothesis that single or repeated exposures to subclinical levels of sarin, particularly under conditions of heat stress, would impair regulation of body temperature and locomotor activity.

## METHODS

**Overall study design.** Rats housed under normal (25°C) or heat-stress (32°C) conditions were exposed nose-only to vehicle or to subclinical doses of sarin gas for 1 h each day for either 1, 5, or 10 days. The rationale for choosing the low and high subclinical doses (0.2 and 0.4 mg/m<sup>3</sup>) has been discussed in a separate paper (Henderson *et al.*, 2002). The choice of the exposure concentrations was based on a literature report of the LC<sub>50</sub> in rats as 220 mg/min/m<sup>3</sup> (Munro *et al.*, 1994). We desired to use exposures that were well below the levels that would cause overt clinical signs of toxicity. Therefore, for our high exposure level, we exposed rats to approximately one-tenth the LC<sub>50</sub> or 0.4 mg/m<sup>3</sup> for 60 min (24 mg/min/m<sup>3</sup>); as a precautionary measure, to be sure we were at subclinical levels, we also exposed rats to 0.2 mg/m<sup>3</sup> for 1 h, as the low-level group. Body temperature and activity were monitored via radiotelemetry in undisturbed rats for 2 days prior to sarin exposures through 28 days postexposure.

**Experimental animals.** Male Fischer 344 rats, 10–13 weeks old, were identified by tail tattoos and housed separately under a 12:12 light–dark cycle with light onset at 6:00 a.m. Water and laboratory rodent chow (Teklad rodent diet W8604) were provided *ad libitum*. Rats were randomized by weight into three exposure groups: control, low sarin, and high sarin. Half of each exposure group was housed at normal (25°C) or heat-stress (32°C) ambient temperature. A pilot experiment was used to determine the ambient temperature sufficient to simulate heat stress. All experimental procedures were approved by the Lovelace Respiratory Research Institute Institutional Animal Care and Use Committee. Lovelace Respiratory Research Institute is fully accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

**Experimental procedures.** Seven days prior to the beginning of each experiment, rats were implanted intraabdominally with miniature battery-operated, temperature-sensitive radio transmitters (VM-FH, Mini-Mitter, Bend, OR). Briefly, rats were anesthetized with halothane, and a 2-cm incision was made through the skin and abdominal muscles. A transmitter was placed in the peritoneal cavity, and the muscle and skin were then separately sutured. Animals were permitted to recover for 7 days and then placed on monitoring boards containing a radio antenna. Output was collected in a personal computer using Datacol 3 software (Mini-Mitter). The animal core temperature is proportional to the signal frequency emitted by the implanted transmitter. Any change in the position of the implanted transmitter relative to the antenna under the cage is recorded as a pulse of activity and accumulated in counts per hour. The frequency emitted by each transmitter was calibrated prior to implantation and at the completion of the studies. Data were included if the transmitter recalibrated within 6 Hz of the original calibration. Thus, 12 rats per group were implanted (total of 96), and data for 8–12 animals per group are reported.

Rats were transported to the nose-only exposure chambers, placed individually in the restrainer attached to each nose-hole of the exposure chamber, and

groups in separate chambers were exposed for 1 h to 0.0, 0.2, or 0.4 mg/m<sup>3</sup> for 1, 5, or 10 days. The exposure chamber and methods for assuring accurate exposure concentrations have been described completely (Henderson *et al.*, 2002).

**Statistical methods.** The objective of the study was to evaluate the effects of sarin exposure on regulation of body temperature under normal and heat-stressed conditions. Activity measurements provided supporting evidence for the observed effects. Three experimental factors were examined: number of days of sarin exposure (1 h per day for 1, 5, or 10 days), sarin concentration (0, 0.2, or 0.4 μg/m<sup>3</sup>), and the temperature of the room where the animals were housed (25 or 32°C).

The rats were monitored for temperature and activity continuously with measurements recorded at 5-min intervals beginning 2 days prior to the first sarin exposure and continuing through 1 month after the last sarin exposure. No measurements were recorded during transport to and from the exposure chambers. Body temperatures during the exposure were monitored by rectal probes in four animals per group (Henderson *et al.*, 2002) and were not included in the statistical analyses in this report.

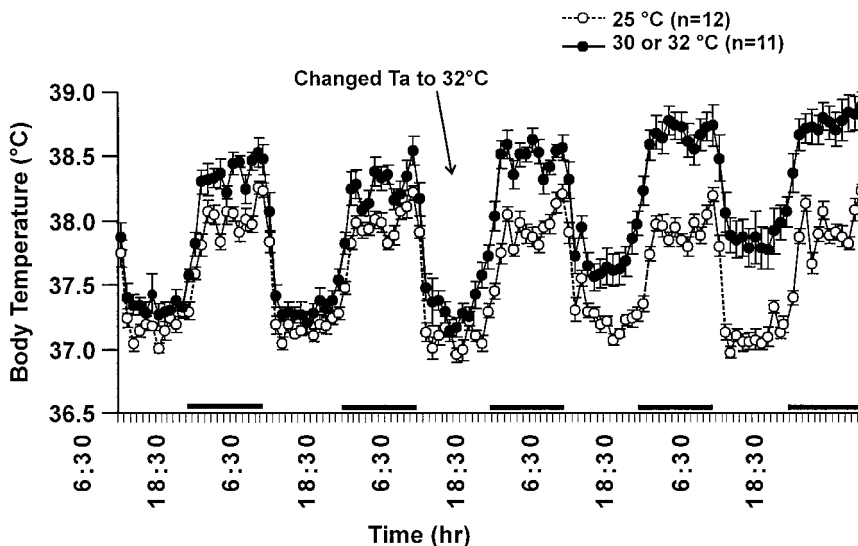
Because of the large quantity of data for each animal (288 temperature and activity measurements per day for up to 30 days), the data were grouped for statistical analysis. First, using all the measurements, the data were checked for unusual temperature values. For any 10-min interval (three measurements) in which the first and third temperature measurements were similar but the second measurement was 2 or more degrees different, the second measurement was replaced with the average of the first and third values. It was not possible to check for unusual activity values by a similar test; however, activities were set to “missing” if the temperature for that time interval was missing.

Once the data were corrected, they were grouped into four intervals: morning (2 a.m. to 10 a.m.; *n* = 96), daytime or light period (10 a.m. to 2 p.m.; *n* = 48), evening (2 p.m. to 10 p.m.; *n* = 96), and night or dark period (10 p.m. to 2 a.m.; *n* = 48). Because of skewness in activity measurements, temperature and activity data within each interval were summarized with a nonparametric parameter: the median (measure of central tendency and analogous to the parametric mean). Thus, the first step of data reduction was to summarize the 288 daily measurements into four daily measurements (the median for each of the four intervals).

Of these measurements, only the night (dark period) and the daytime (light period) intervals were analyzed statistically. Animals are expected to be most active during the dark period and least active during the light period. The morning and evening intervals represent periods of transition. These periods of transition were assumed to have greater levels of natural variability, which would make detection of differences associated with the experimental factors more difficult. Within the two selected time intervals, the median temperature was considered to be the most sensitive variable to sarin exposure. The median activity was considered to provide supporting evidence for the effects on body temperature.

The daily measurements were made for a month after the exposures. Because the statistical approach was designed to compare the effects of different lengths of sarin exposure, only a few of the days were included in the analysis. Specifically, measurements made in the first preexposure day, the first day of sarin exposure, the last day of sarin exposure (equal to the first day in the 1-day exposure experiment), the first day of recovery, and a time point late in the recovery period were used. Although the recovery period was intended to last 30 days, in one group of animals the actual measurement period was 21 days due to technical recording difficulties during the last week of recovery. To determine the appropriate method to summarize the recovery information, plots were drawn for each animal to determine any linear or nonlinear trends in temperature or activity over time. None was observed, and it was decided that the recovery information would be best described by using the day 20 data for the statistical analysis.

The data were analyzed in three separate repeated measures analyses of variance. First, to examine the acute effects of sarin exposure, the night measurements for the first preexposure day and for the first day of sarin exposure were analyzed as the vector of dependent variables. It would be



**FIG. 1.** Core temperature of rats housed in thermoneutral ambient temperature (25°C) or in two levels of heat stress (30°C and 32°C). Data points represent hourly means  $\pm$  SEM. Dark bars indicate hours of darkness in the 12:12 light cycle. Arrow indicates when the ambient temperature ( $T_a$ ) of rats housed at 30°C was elevated to 32°C.

expected that there should be no difference associated with the number of days of sarin exposure, as all animals were exposed only for 1 day at that point. The dark period measurements were used as the animals were being exposed during the light period. Not only are there fewer recorded measurements during exposure days, but also the stress of the exposure and the transport to and from the exposure rooms may be expected to affect temperature and activity levels in ways that would not be associated with sarin itself but would confound interpretation of results.

Second, to examine the long-term effects of sarin exposure, a vector was constructed with dark period data for the first preexposure day, the last day of sarin exposure, the first day postsarin exposure, and day 20 postsarin exposure. For the reasons given above, this vector of time points was examined only for the dark period data.

Finally, the long-term effects of sarin exposure on temperature and activity during the light period were examined with a dependent variable profile including the first preexposure day, the first day postexposure, and day 20 postexposure. Light period measurements *during* sarin exposures were not collected by telemetry and were not included in the statistical analyses.

The median temperature and activity values were used as the dependent variables for the repeated measures analyses described above.

The statistical analysis consisted of six repeated measures analyses: the three repeated measures designs described above to assess acute and long-term effects, performed for each of the two analysis variables (median activity and median temperature). For each repeated measures analysis, the independent variables were number of days of sarin exposure (days\_exp), sarin concentration (conc), temperature of the rooms in which the animals were housed (rm\_temp), and all possible two-way and three-way interactions. Statistical significance ( $p < 0.05$ ) was assessed using the Hotelling–Lawley trace. A multivariate contrast was also performed comparing two adjacent time points (e.g., the preexposure data compared to the last day of sarin exposure; the last day of sarin exposure compared to the first postexposure day, and so on). If there were any statistically significant multivariate effects, the univariate ANOVAs for each time point were examined. The same independent variables were used including all possible interactions. Subtesting of statistically significant ANOVA effects (assessed by  $F$  tests,  $p < 0.05$ ) was performed as needed by  $t$  test. Because of the step-down nature of the analysis as well as the relatively small sample sizes for some of the interactions, no controlling for multiple comparisons was performed.

## RESULTS

### Pilot Study

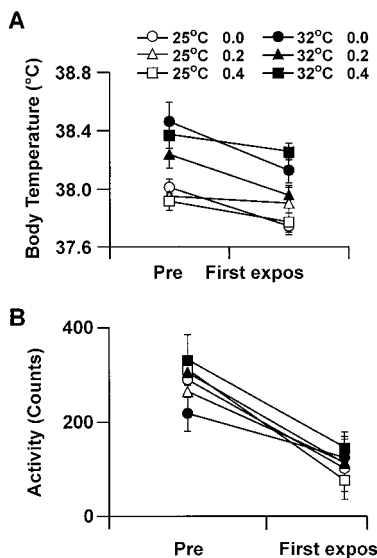
An ambient temperature of 32°C elevated core temperature in unexposed rats by approximately 1°C relative to animals housed at thermoneutral ambient (Fig. 1), and this ambient temperature was used to simulate a mild chronic heat stress during the subsequent experiments involving exposures. Body temperatures during the 1-h inhalation exposures were monitored by rectal probes in the restrained rats and were maintained at 37°C for rats housed at 25°C and at 38°C for rats housed at 32°C (Henderson *et al.*, 2002).

### Acute Effects

Animals housed in the 32°C room had statistically higher body temperatures during the dark periods than animals housed in the 25°C room on the preexposure day. Although there was a trend toward lower temperature after the first exposure (see Fig. 2A), this drop in temperature did not attain statistical significance. There was also a trend to lower activity (Fig. 2B) in the dark period following the first exposure in all groups. This trend was not due to differences in housing temperature or type of exposure (air or sarin).

### Exposure and Recovery Effects (Dark Period)

Neither effects due to concentration of sarin nor any interactions involving sarin were found in the multivariate analysis. As can be observed in Fig. 3A, the body temperature of rats housed at 32°C was higher by approximately 0.4° during the dark period relative to rats housed at 25°C over the observation



**FIG. 2.** Acute effects of sarin exposure. Body temperature (A) and locomotor activity (B) during the middle part of the dark period in rats before (Pre) and after (First expos) one inhalation exposure of either air or sarin gas. Each data point represents the mean  $\pm$  SEM of the median dark period temperature for 8–11 rats.

period. There were no consistent effects of the study parameters on activity (Fig. 3B).

#### Exposure and Recovery Effects (Light Period)

No effects that might be attributed to sarin concentration were observed for body temperature or activity during the light period. Housing at 32°C was associated with an increased body temperature during the light period relative to housing at 25°C prior to sarin exposure but not at the subsequent study time points (Fig. 4A). In general, the effects of the study parameters on body temperature during the light period were not as pronounced as those during the dark period. This might be expected as the animals are more active during the dark than during the light (compare Figs. 3B and 4B) and have a lower normal body temperature (see Fig. 1). Activity during the light period was generally low, but somewhat higher in the animals housed at 32°C than at 25°C (Fig. 4B).

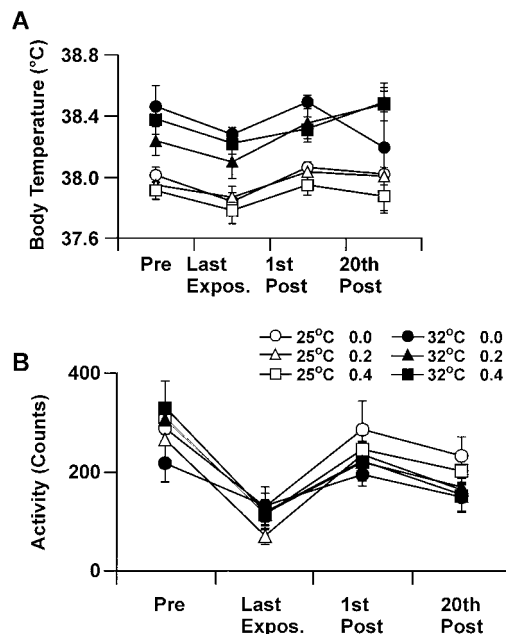
### DISCUSSION

Inhalation of sarin gas at two subclinical levels did not affect body temperature acutely in a biologically meaningful manner after the first exposure or after 5 or 10 repeated exposures either at thermoneutral ambient temperature or during chronic heat stress. The experimental handling of the rats to expose them to the gases produced a slight decrease in expected body temperature during the night following the first handling/exposure stress, and this effect persisted as long as the night following the last handling/exposure stress. However, sarin gas did not exacerbate this disturbance in temperature relative to

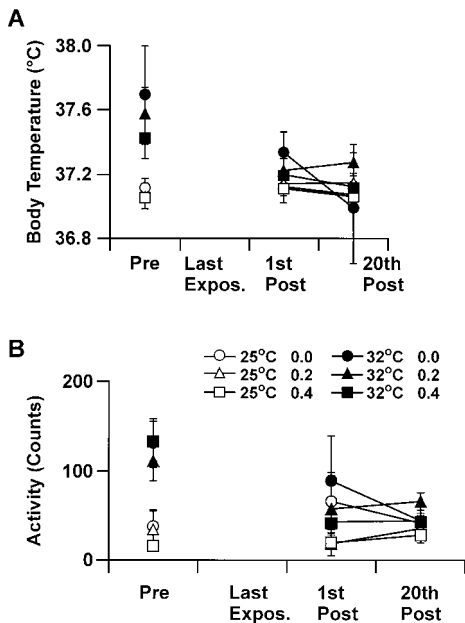
the no-sarin control rats at either ambient temperature. These data are consistent with the hypothesis that the subclinical levels of sarin, which were sufficient to reduce red blood cell cholinesterase activity but not plasma cholinesterase activity (Henderson *et al.*, 2002), were below the threshold necessary to significantly alter body temperature regulation even under conditions of chronic heat stress. Others have shown that high levels of organophosphate cholinesterase inhibitors can cause hyperthermia or hypothermia, depending upon experimental conditions, which may require days to resolve (Gordon, 1996). Our data suggest that subclinical levels have minimal effects on temperature regulation under our observation conditions.

Although one might have expected decreased activity in rats housed under heat stress, the increased activity observed in the rats housed under heat stress (32°C) during the light period in the early part of the observations was similar to the increased activity observed by others under acute conditions of slightly higher (36°C) ambient heat stress (Chuang and Lin, 1994). Data collected under a different heating paradigm by Galina *et al.* (1983) also showed that milder temperature elevations increased activity in rats, whereas higher elevations decreased activity. It may be that the lower activity (see Fig. 4B) and the lower body temperatures (see Fig. 4A) during the light period in the later part of our observations indicate heat acclimatization during the less active part of the 24-h day.

In conclusion, our data showed that exposure of rats to low levels of sarin, regardless of whether they were undergoing



**FIG. 3.** Effects of repeated exposure to sarin during the dark period. Body temperature (A) and locomotor activity (B) during the middle part of the dark period in rats before (Pre) and after the last exposure (Last expos) of either air or sarin gas and on the night 1 and night 20 postexposure. Each data point represents the mean  $\pm$  SEM of the median dark period temperature for 8–11 rats.



**FIG. 4.** Effects of repeated exposure to sarin during the light period. Body temperature (A) and locomotor activity (B) during the middle part of the light period in rats before (Pre) exposure to either air or sarin gas and on the night 1 and night 20 postexposure. The data were not collected by telemetry during the last exposure (which occurred during the light period) and thus are missing from the graph. Each data point represents the mean  $\pm$  SEM of the median light period temperature for 8–11 rats.

heat stress, did not significantly alter their temperature regulation or locomotor activity. The rats housed at 32°C showed signs consistent with mild heat stress, but this stressor did not exacerbate their response to sarin gas relative to those rats housed in their thermoneutral zone. To the extent that thermoregulation is an indicator of neuroimmune function and locomotor activity can indicate fatigue, these data do not support the theory that subclinical exposure to nerve gas under conditions of heat stress may have resulted in these clinical symptoms of the Gulf War Syndrome.

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tory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH publication 86-23, revised 1985).

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