Presentation 4 – Rogene Henderson

EFFECTS OF INHALATION EXPOSURE TO LOW LEVELS OF SARIN IN FISCHER 344 RATS

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THE QUESTION

What are the potential health effects from single or repeated exposure to subclinical (acutely asymptomatic) levels of sarin? Does heat stress potentiate those health effects?

PRELIMINARY STUDY

- Purpose:
  - To determine if the exposure levels chosen on the basis of literature reports were sub-clinical.
- Approach:
  - Exposed four rats/group to 0, 0.2, 0.4 or 0.8 mg/m^3 sarin for 1 hr.
- Results:
  - No observed abnormal behaviors (salivation, lacrimation, red eye discharge, sneezing, excess face rubbing, excess urination, excess defecation, diarrhea, muscle tremors, muscle weakness, paralysis)
- Conclusion:
  - Exposure levels chosen are sub-clinical

EXPERIMENTAL DESIGN

- Animals:
  - Male, F344 rats, 10–11 weeks old
- Exposures:
  - Nose-only to 0, 0.2 or 0.4 mg/m^3 of sarin for 1 hour/day, for 1, 5 or 10 days
- Heat stress:
  - Exposures were done at either normal (25°C) temperature or under heat stress (32°C)
- Sacrifice times:
  - 1 day after exposure to observe acute effects
  - 30 days after exposure to determine if any effects are persistent
- Observations:
  - Body weights, activity patterns, body temperature, histopathology, apoptotic cells in the brain, brain cytokine levels (IL-1β, TNFα, IL-6), densities of brain muscarinic receptor sites
  - Limited observations on effects on the immune system

METHODS

- Exposures:
  - Nose-only, inside a glove box. Monitored by minicam (GC/FPD). Exhaust scrubbed by sodium hypochlorite bubbler.
- Pulmonary function during exposure:
  - Plethysmography exposure tubes that hold a Fleisch pneumotach connected to a validyne differential pressure transducer (DP45). The signal from the transducer indicates the volume displacement caused by breathing.
- Body temperature during exposure:
  - Rectal probes.
- Body temperature and activity after exposure:
  - Surgically implanted biotelemetry devices transmitted information to activity boards under the rat cages.

SARIN EXPOSURE LEVELS (mg/m^3, $\bar{X}$ ± SD)[a]

	Normal Temperature		High Temperature	
Days of	Low Level	High Level	Low Level	High Level
1	0.22 ± 0.04	0.38 ± 0.06	0.21 ± 0.03	0.42 ± 0.06
5	0.19 ± 0.02	0.37 ± 0.02	0.18 ± 0.03	0.37 ± 0.02
10	0.20 ± 0.01	0.42 ± 0.03	0.21 ± 0.01	0.42 ± 0.02

[a]The $\bar{X}$ ± SD values for the 1 day exposure were based on 15 measurements taken every 4 min during the 1 hr exposures. The values for repeated exposures are the mean and SD of the daily exposure values.

BODY TEMPERATURE DURING EXPOSURE

($\bar{X}$ ± SD, n = 4 animals)

	Control	0.2 mg/m^3	0.4 mg/m^3
Normal Temperature	36.5 ± 0.8	36.5 ± 0.8	36.8 ± 0.9
Heat Stress	37.7 ± 0.5	38.0 ± 0.7	38.0 ± 0.7

BODY WEIGHTS

(g, $\bar{X}$ ± SE)

	Before Exposure	End of Exposure	1 Month After Exposure
Sarin Exposure Group	n = 24	n = 12	n = 12
1–Day Exposure (NT)			
Control	216 ± 1.6	227 ± 2.9	272 ± 3.6
0.2 mg/m^3	213 ± 1.8	225 ± 1.7	270 ± 4.4
0.4 mg/m^3	213 ± 1.8	223 ± 1.8	267 ± 4.4
1–Day Exposure (HT)			
Control	222 ± 1.0	218 ± 1.9[a]	239 ± 4.9[a]
0.2 mg/m^3	220 ± 1.3	222 ± 2.1	247 ± 2.4[a]
0.4 mg/m^3	221 ± 1.2	225 ± 1.9	247 ± 2.3[a]

NT = normal temperature (25°C); HT = high temperature (32°C)
[a]Weight gain differs from NT, $P \leq 0.05$.

BODY WEIGHTS

(g, $\bar{X}$ ± SE)

Sarin Exposure Group	Before Exposure	End of Exposure	1 Month After Exposure
	n = 24	n = 12	n = 12
5–Day Exposure (NT)			
Control	213 ± 1.2	231 ± 1.3	281 ± 3.0
0.2 mg/m³	215 ± 1.6	231 ± 0.6	282 ± 5.4
0.4 mg/m³	214 ± 1.5	224 ± 3.0	279 ± 4.4
5–Day Exposure (HT)			
Control	214 ± 1.6	218 ± 1.4*	259 ± 4.2*
0.2 mg/m³	214 ± 1.8	220 ± 2.2*	260 ± 3.2*
0.4 mg/m³	214 ± 1.4	220 ± 0.6	253 ± 2.1*

NT = normal temperature (25°C); HT = high temperature (32°C)
*Weight gain differs from NT, P ≤ 0.05.

BODY WEIGHTS

(g, $\bar{X}$ ± SE)

Sarin Exposure Group	Before Exposure	End of Exposure	1 Month After Exposure
	n = 24	n = 12	n = 12
10–Day Exposure (NT)			
Control	222 ± 3.2	231 ± 2.7	295 ± 3.6
0.2 mg/m³	227 ± 1.9	229 ± 2.9	290 ± 3.2
0.4 mg/m³	224 ± 1.7	231 ± 3.5	296 ± 3.4
10–Day Exposure (HT)			
Control	210 ± 1.7	214 ± 2.5	242 ± 3.6*
0.2 mg/m³	210 ± 1.4	218 ± 2.4	248 ± 2.6*
0.4 mg/m³	211 ± 1.0	215 ± 1.6	241 ± 2.9*

NT = normal temperature (25°C); HT = high temperature (32°C)
*Weight gain differs from NT, P ≤ 0.05.

PULMONARY FUNCTION

(during exposure; $\bar{X}$ ± SE, n = 4)

Sarin Exposure Group	Frequency (breaths/min)	Tidal Volume ml	Minute Volume ml/min
1–Day Exposure (NT)			
Control	159 ± 16	1.5 ± 0.08	236 ± 23
0.2 mg/m³	169 ± 1	1.6 ± 0.12	267 ± 19
0.4 mg/m³	149 ± 1	1.6 ± 0.07	231 ± 11
1–Day Exposure (HT)			
Control	155 ± 7	1.5 ± 0.02	238 ± 13
0.2 mg/m³	149 ± 5	1.5 ± 0.03	223 ± 10
0.4 mg/m³	156 ± 5	1.6 ± 0.06	242 ± 13

NT = normal temperature (25°C); HT = high temperature (32°C)

PULMONARY FUNCTION

(during exposure; $\bar{X}$ ± SE, n = 4)

Sarin Exposure Group	Frequency (breaths/min)	Tidal Volume ml	Minute Volume ml/min
5–Day Exposure (NT)			
Control	160 ± 6	1.5 ± 0.07	236 ± 20
0.2 mg/m³	170 ± 3	1.5 ± 0.07	267 ± 19
0.4 mg/m³	165 ± 3	1.5 ± 0.08	252 ± 9
5–Day Exposure (HT)			
Control	166 ± 7	1.6 ± 0.13	258 ± 15
0.2 mg/m³	174 ± 9	1.5 ± 0.07	264 ± 23
0.4 mg/m³	172 ± 6	1.6 ± 0.06	269 ± 10

NT = normal temperature (25°C); HT = high temperature (32°C)

PULMONARY FUNCTION

(during exposure; $\bar{X} \pm SE$, n = 4)

Sarin Exposure Group	Frequency (breaths/min)	Tidal Volume ml	Minute Volume ml/min
10–Day Exposure (NT)			
Control	160 ± 2	1.5 ± 0.02	238 ± 5
0.2 mg/m³	154 ± 2	1.6 ± 0.02	247 ± 6
0.4 mg/m³	164 ± 2	1.5 ± 0.02	253 ± 6
10–Day Exposure (HT)			
Control	142 ± 2	1.6 ± 0.02	222 ± 4*
0.2 mg/m³	149 ± 2	1.6 ± 0.02	232 ± 4*
0.4 mg/m³	144 ± 2	1.6 ± 0.02	230 ± 4*

NT = normal temperature (25°C); HT = high temperature (32°C)

BLOOD CHOLINESTERASE IN RATS

Sarin Exposure (mg/m³)	1 Day of Exposure (n = 12)	
	RBC ChE ($\bar{X} \pm SE$)	Plasma ChE ($\bar{X} \pm SE$)
0	1.553 ± 0.040	0.236 ± 0.008
0.2	1.437 ± 0.040*	0.202 ± 0.006
0.4	1.387 ± 0.034*	0.189 ± 0.008

*Differs from control, $p \leq 0.05$

BLOOD CHOLINESTERASE IN RATS

Sarin Exposure (mg/m³)	5 Days of Exposure (n = 10-11)	
	RBC ChE ($\bar{X} \pm SE$)	Plasma ChE ($\bar{X} \pm SE$)
0	1.946 ± 0.064	0.162 ± 0.006
0.2		
0.4	0.804 ± 0.020*	0.140 ± 0.007†

*Differs from control, $p \leq 0.05$
†Differs from control, p = 0.055

BLOOD CHOLINESTERASE IN RATS

Sarin Exposure (mg/m³)	10 Days of Exposure (n = 10-11)	
	RBC ChE ($\bar{X} \pm SE$)	Plasma ChE ($\bar{X} \pm SE$)
0	1.681 ± 0.042	0.231 ± 0.011
0.2	1.125 ± 0.040*	0.227 ± 0.004
0.4	0.618 ± 0.037*	0.220 ± 0.012

*Differs from control, $p \leq 0.05$

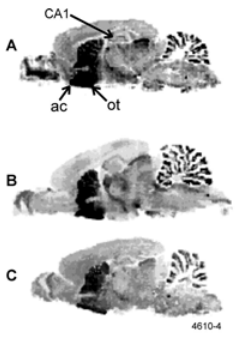
BRAIN CHOLINESTERASE ACTIVITY IN 5-DAY EXPOSED RATS, 1 DAY AFTER EXPOSURES

(U ChE activity/µg protein; $\overline{X} \pm$ SE, n = 3)

Sarin Exposure (mg/m^2)	NT (25°C)	HT (32°C)
0	5.41 ± 0.16	5.26 ± 0.06
0.2	5.15 ± 0.10	5.11 ± 0.15
0.4	5.66 ± 0.31	5.15 ± 0.20

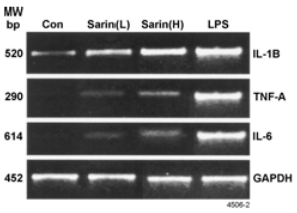
BRAIN AChE BY HISTOCHEMICAL STAINING

- AChE reduced in cerebral cortex, striatum and olfactory bulb.
- Loss of laminar pattern of AChE in hippocampus over CA1 and CA3 sectors.
- Diminished forebrain, but not brain stem AChE.



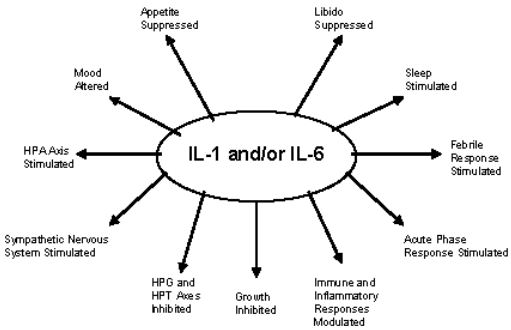
CYTOKINES IN BRAIN

IL-1β, IL-6 and TNF-α were all induced in a dose-dependent manner in the brains of rats exposed to sarin for 5 days, but not in rats exposed for 1 day.



Sarin induces the expression of IL-1β, IL-6, and TNF-α in the brain

CYTOKINES AND THE NEUROIMMUNE RESPONSE

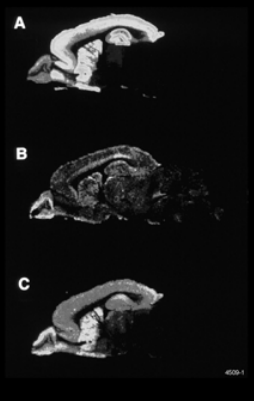


MUSCARINIC ACETYLCHOLINE RECEPTOR SITES (Coupled to G Proteins)

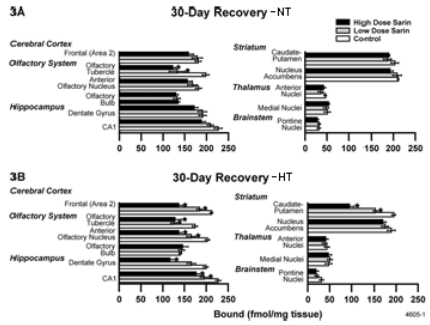
M1 – Cerebral cortex, forebrain, telecephalic structures, paralimbic cortical areas

M2 – All sensory and motor areas, cerebral cortex

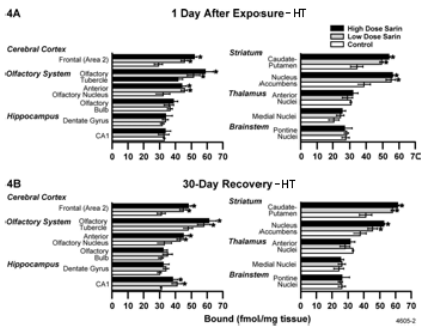
M3 – Forebrain, telecephalic structures, auditory areas of temporal lobe, hypothalamus



M1 RECEPTOR SITES



M3 RECEPTOR SITES



END OF EXPOSURE

	M1	M2	M3
Normal Temperature	no change	no change	no change
High Temperature	no change	no change	↑

frontal lobe
olfactory
tubercle
striatum

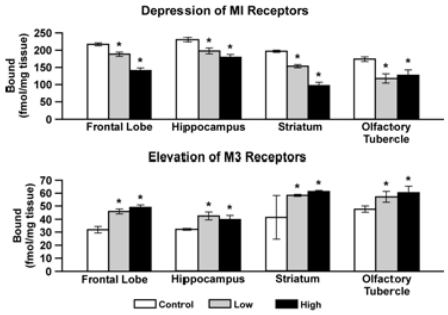
RAT BRAIN

Areas of increased M3 receptor sites.

THIRTY DAYS AFTER FIVE REPEATED 1 HR/DAY EXPOSURES

	M1	M2	M3
Normal Temperature	↓ olfactory tubercle	no change	no change
High Temperature	↓ Frontal Area 2 Olfactory tubercle Olfactory nucleus Hippocampus Striatum	no change	↑ Frontal Area 2 Olfactory tubercle Olfactory nucleus Hippocampus Striatum

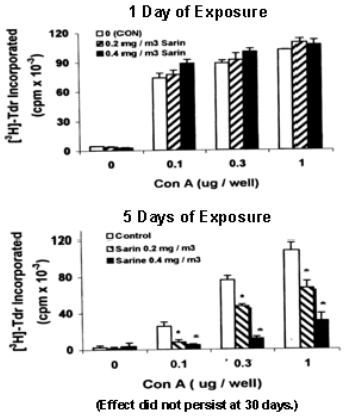
THIRTY DAYS AFTER FIVE REPEATED 1 HR/DAY EXPOSURES
(Heat Stress Conditions)



DOES SARIN AFFECT THE IMMUNE SYSTEM?

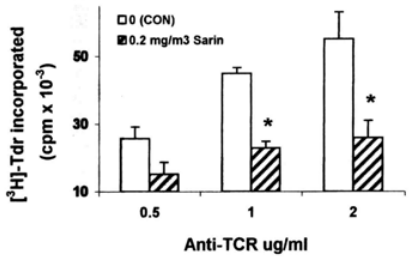
- Bidirectional communication between the brain and the immune system.
- Lymphoid tissues are innervated by both sympathetic and parasympathetic nerves. The function of these innervations is not clear.
- Cholinergic agents, including organophosphates, affect some immune parameters.

SARIN EXPOSURE INHIBITED T-CELL RESPONSE TO MITOGEN



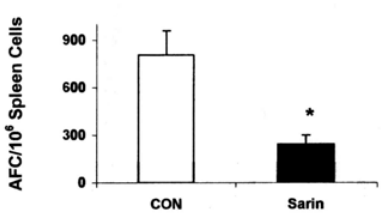
(Effect did not persist at 30 days.)

SARIN SUPPRESSES PROLIFERATIVE RESPONSE OF T-CELLS TO ANTIGEN

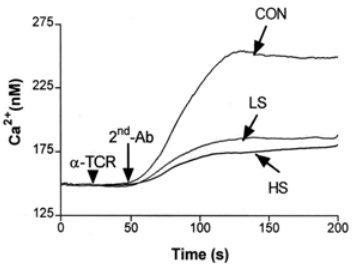


SARIN SUPPRESSES ANTIBODY RESPONSE TO SHEEP RED BLOOD CELLS

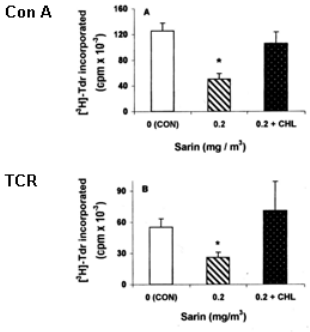
(0.4 mg/m³ sarin, 1 h/day, 5 days)

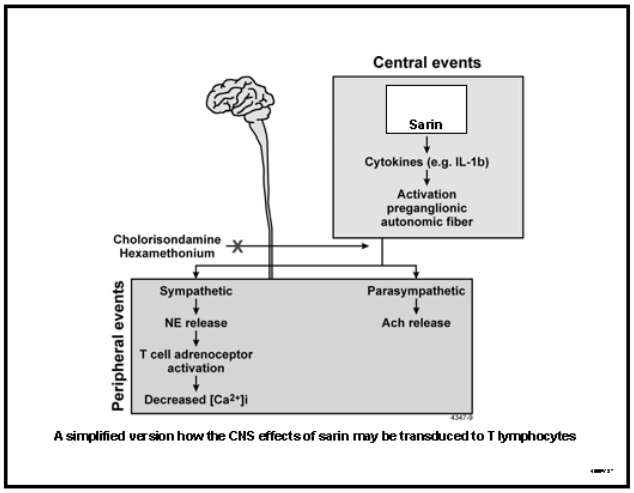


SARIN SUPPRESSES THE CALCIUM INCREASE THAT ACCOMPANIES Ag-MEDIATED T-CELL ACTIVATION

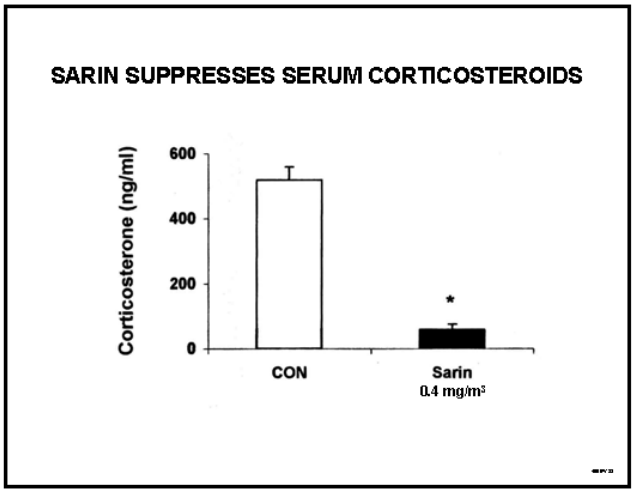


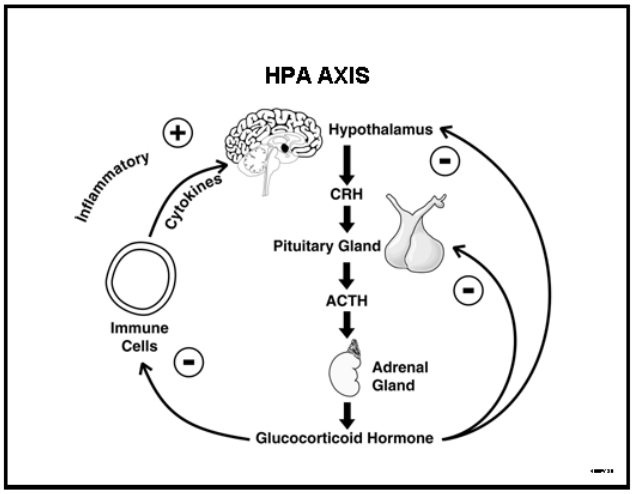
A GANGLIONIC BLOCKER ATTENUATES SARIN SUPPRESSION OF T-CELL PROLIFERATION

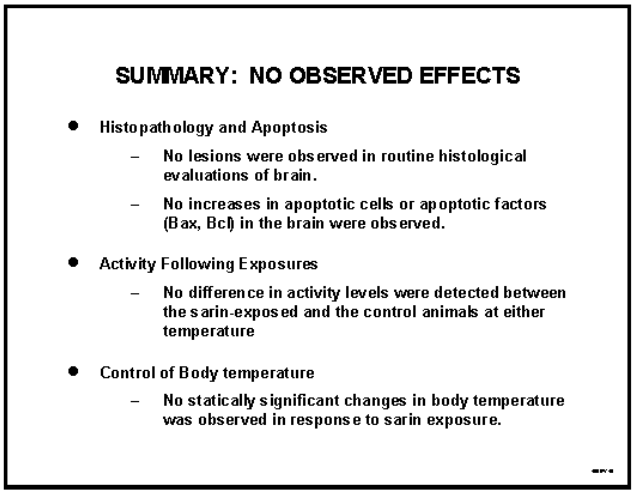




A simplified version how the CNS effects of sarin may be transduced to T lymphocytes







OBSERVED EFFECTS

- Subclinical exposures to sarin induced the expression of brain cytokines involved in neuroimmune responses.
- Combined exposure to sarin and heat stress caused a statistically significant increase in M3 receptor sites in the olfactory and adjacent areas of the brain that persisted for 30 days. There was a delayed (30 day) increase in M3 receptor sites in the hippocampus, a region of the brain important for memory and cognitive function.
- Sarin alone caused a decrease in M1 receptor sites in the olfactory tubercle. Sarin plus heat stress caused a delayed decrease in M1 receptor sites in the cerebral cortex, the olfactory region, the striatum and the hippocampus.

OBSERVED EFFECTS

- Repeated subclinical exposures to sarin inhibited antibody formation and antigen-mediated T cell proliferation.
- Changes in the T cell function may reflect impaired antigen-receptor-mediated signaling in T cells.
- Immunosuppression is not the result of increased CORT production; however, sarin may affect the immune system via the autonomic nervous system.
- Sarin and other cholinergic agents may decrease CORT levels, and serum CORT level may serve as biomarker for cholinergic exposure.

CONCLUSIONS

- Repeated subclinical exposure to sarin results in suppression of immune responses.
- Repeated subclinical exposure to sarin, especially under heat stress, results in persistent, as well as delayed, alterations in the density of acetyl choline receptor sites in areas of the brain responsible for memory and cognitive function.

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3. Conn, C. A., K. Dokladny, M. G. Ménache, E. B. Barr, W. Kozak, A. Kozak, M. Wachulec, K. Rudolph, M. J. Kluger and R. F. Henderson: Effects of Sarin on Temperature and Activity of Rats as a Model for Gulf War Syndrome Neuroregulatory Functions. *Toxicol. Appl. Pharmacol. 184(2)*: 77-81, 2002.