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Evaluation of Candidate Adenosine Receptor Agonists as Neuroprotective Countermeasures for Soman Intoxication

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A thesis in partial fulfillment of the requirements for the degree of Master of Arts in Experimental Psychology.

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Abstract

Soman, an organophosphorus (OP) compound, disrupts nervous system function through inactivation of acetylcholinesterase (AChE), the enzyme that breaks down acetylcholine at synapses. Left untreated, a state of prolonged seizure activity (*status epilepticus*, SE) is induced, causing widespread neuronal damage and associated cognitive and behavioral impairments. Previous research demonstrated that therapeutic stimulation of A1 adenosine receptors (A1ARs) can prevent or terminate soman-induced seizure. Here, we examined the ability of three potent A1AR agonists to provide neuroprotection and, ultimately, prevent observable cognitive and behavioral deficits following exposure to soman. Sprague Dawley rats were challenged with a seizure-inducing dose of soman (1.2 x LD_{50}) and treated 1 minute later with one of the following A1AR agonists: (6)-Cyclopentyladenosine (CPA), 2-Chloro-N6-cyclopentyladenosine (CCPA) or (±)-5'-Chloro-5'-deoxy-ENBA (cdENBA). An active avoidance shuttle box task was used to evaluate locomotor responses to aversive stimuli at 3, 7 and 14 days post-exposure. Animals treated with CPA, CCPA or cdENBA demonstrated a higher number of avoidance responses and a faster reaction to the aversive stimulus than the soman/saline control group across all three sessions. Findings suggest that A1AR agonism is a promising neuroprotective countermeasure, capable of preventing the long-term deficits in learning and memory that are characteristic of soman intoxication.

Keywords: Adenosine receptor agonist, antidote, behavior, neuropathology, organophosphate, seizure activity, shuttle box, soman

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Evaluation of Candidate Adenosine Receptor Agonists as Neuroprotective Countermeasures for Soman Intoxication

Organophosphorus agents (OPs), such as soman, are irreversible cholinesterase inhibitors. Acute exposure to these compounds can result in rapid incapacitation or death at high doses (Sidell & Borack, 1992). Because of their unique chemical properties, OPs bind to acetylcholinesterase (AChE), rendering the enzyme incapable of hydrolyzing acetylcholine (ACh) in the cholinergic synapses and neuromuscular junctions. Subsequent accumulation of ACh leads to overstimulation of the affected neurons. The peripheral effects of excess systemic ACh include observable toxic signs (e.g., miosis, lacrimation, salivation, fasciculations, tremors and convulsions), as well as life-threatening cardiovascular and respiratory distress. Simultaneous progression of the cholinergic crisis within the central nervous system (CNS) ultimately induces a state of unremitting seizure known as *status epilepticus* (SE). Unmitigated OP-induced SE is associated with widespread neuronal damage, and concomitant cognitive and behavioral deficits (Petras, 1994; McDonough & Shih, 1997).

Because OPs undergo the process of aging (when the chemical bond between OP and AChE becomes permanent), the temporal window for delivery of effective treatment is narrow. Soman is often considered one of the most lethal OPs as it ages in mere minutes (Sun, Chang, Shau, Huang, & Chou, 1979). While this further complicates treatment efforts, it also makes soman the ideal candidate for challenging potential pharmaceutical interventions for OP intoxication.

Current Treatment Regimens

Preventative treatments like pyridostigmine bromide (PB; a reversible cholinesterase inhibitor) may be administered when exposure to fast-acting OPs (e.g., soman) is anticipated. PB temporarily binds to peripheral AChE, proactively shielding a percentage of the enzyme from being inactivated during OP exposure (Kluwe, Chinn, Feder, Olson & Joiner, 1987). Once signs of OP poisoning are present, however, PB treatment is no longer effective and may even exacerbate toxicity (Sidell & Borack, 1992).

Following exposure, immediate survival is dependent upon controlling the peripheral effects of OPs (i.e., respiratory and cardiovascular distress). Atropine (a competitive antagonist of ACh receptors) is typically delivered in conjunction with pralidoxime chloride (2-PAM; an oxime intended to reactivate AChE activity). Though both drugs have demonstrated efficacy in ameliorating peripheral symptoms, their combination will not prevent the onset of seizure activity (Shih, Rowland, & McDonough, 2007). As a result, the current standard of care also includes reactionary administration of anticonvulsants (e.g., diazepam). Unfortunately, OP-induced SE can quickly become refractory to this doctrinal intervention, leaving those exposed vulnerable to developing neuropathology and, consequentially, long-term cognitive and behavioral impairments (Petras, 1994; McDonough, Dochterman, Smith & Shih, 1995; Shih, Duniho, & McDonough, 2002). The suboptimal CNS protection offered by the current treatment regimen warrants continued exploration of therapeutic targets capable of both preventing and rapidly terminating OP-induced seizure activity. Toward that end, this study evaluated the therapeutic augmentation of adenosine signaling as a potential alternative neuroprotective countermeasure for OP poisoning.

Adenosine Receptor Agonists as a Neuroprotective Countermeasure

Adenosine (ADO), an extracellular signaling molecule produced primarily from the metabolism of adenosine triphosphate (ATP), is involved in modulating a range of physiological functions throughout the brain and periphery. ADO's diverse effects on different organ systems can be attributed to its action upon four ubiquitously expressed receptor subtypes. ADO A1 receptor (A1AR) stimulation, in particular, results in a profound inhibition of synaptic transmission that has been exploited for its diagnostic and therapeutic applications including the prevention and suppression of epileptic seizure activity (Haas & Selbach, 2000; Weltha, Reemmer & Boison, 2018).

While the A1AR agonist (6)-Cyclopentyladenosine (CPA) has demonstrated potential for attenuating seizure activity and promoting survival in rats acutely poisoned with soman and sarin (van Helden, Groen, Moor, Westerink, & Bruijnzeel, 1998; Bueters, Groen, Danhof, Ijzerman & van Helden, 2002), off-target side effects (e.g., hypotension and bradycardia (Schindler et al., 2005)) initially slowed clinical adaptation. Through renewed interest and continued exploration, A1AR stimulation has been better optimized as a countermeasure for OP exposure. Alternative routes of administration, as well as compounds with greater A1AR affinities (e.g., CCPA and cdENBA), have since been investigated with promising results (Thomas & Shih, 2014; Thomas, Wegener & Shih, 2018). While intraperitoneally administered CPA (60mg/kg) was found to reduce the incidence of seizure onset and promote survival (8% seizure, 83% survival, $N = 12$), two additional A1AR agonists, 2-Chloro-N6-cyclopentyladenosine (CCPA; 36mg/kg) and (±)-5'-Chloro-5'-deoxy-ENBA (cdENBA; 62mg/kg), produced similar results (17% seizure, 75% survival, $N = 12$, and 8% seizure, 83% survival, $N = 12$, respectively) with

less severe ADO-induced side effects (e.g., sedation, hypothermia, bradycardia; Thomas et al., 2018).

Avoidance Learning following OP Exposure

Although promoting survival is the immediate goal for counteracting OP intoxication, the long-term pathological sequelae of exposure must also be considered and minimized. It is well-established that the amygdala, hippocampus, piriform cortex, and thalamus consistently exhibit neuronal dysfunction and destruction following onset of soman-induced seizure (Petras, 1994; Shih et al., 2002, Thomas et al., 2018). These areas are believed to play a critical role in multiple aspects of learning and memory.

Animal models suggest that emotional learning and memory are disrupted when neurons in the amygdala sustain damage, as evidenced by abnormalities in the learning and retention of various types of avoidance behavior (Kaada, Andersen, & Jansen, 1954; Maren, 1999; Delgado, Jou, Ledoux & Phelps, 2009). An inability to avoid threatening situations or stimuli is indicative of abnormal psychological functioning and environmental maladaptation (Delgado et al., 2009; Krypotos, Effting, Kindt & Beckers, 2015). Conversely, excessive avoidance is a core feature of all anxiety disorders as defined by The Diagnostic and Statistical Manual of Mental Disorders (DSM-5; American Psychiatric Association, 2013). Atypical hippocampal functioning is also implicated in the development of anxiety disorders (Cominski, Jiao, Catuzzi, Stewart & Pang, 2014). This region is believed to be especially important for processing spatial and contextual information used to facilitate employment of strategic responses in avoidance learning (Black, Nadel, & O'Keefe, 1977; Jarrard, 1993). Studies show that the thalamus is conjointly involved in navigating tasks that rely on spatial memory (Warburton, Baird,

Morgan, Muir & Aggleton, 2001). As a result, thalamic lesions may impact the ability for rodents to learn conditioned avoidance responses (Thompson, 1963; van Groen, Kadish & Wyss, 2002). The piriform cortex is predominantly involved in the perception of smell, a function that is fundamental for sensory memory and habituation in rats (Wilson, 2009). Injury to the periamygdaloid piriform cortex is known to produce impairment in the acquisition of avoidance learning (Grossman, Grossman, & Walsh, 1975).

Because the amygdala, hippocampus, piriform cortex and thalamus are involved in the cognitive processes associated with learning and memory, those exposed to OP can be presumed to suffer from cognitive alterations when neurons from these regions inevitably suffer damage. Shih, Guarisco, Myers, Kan, and McDonough (2011) reported such deficits in learning ability among guinea pigs that experienced OP-induced SE. At 24 hours post-exposure, the animals demonstrated diminished avoidance acquisition and performance as evaluated by a shuttle box assay (a task that measures the ability to associate and respond to a cue signaling an impending aversive stimulus).

Other studies have documented long-term learning deficits in animals following OP-induced SE, as well. At 90 days after soman-induced seizure onset, mice display reduced success in the Morris water maze (a navigation task that examines an animal's ability to recall the location of a platform hidden under the surface of the water; Filliat et al., 1999). These findings were confirmed in rats tested 60 days after soman-induced seizure onset (Joosen et al., 2009). Animals with a diminished ability to effectively search for and locate the platform demonstrate inadequacies in both spatial learning and memory that would presumably affect avoidance learning, as well.

Statement of the Problem

As the ultimate goal of OP countermeasure research is to identify therapeutic regimens capable of effectively treating humans, it is crucial to address the entire scope of the damage sustained during exposure. Previous rodent studies have indicated that compounds targeting A1AR may prevent seizure, promote short-term survival, and offer protection from structural damage in key brain regions. These physiological measures of A1AR agonist-treatment efficacy are encouraging but do not address the behavioral implications of exposure or treatment. To maximize clinical relevance, the efficacy of potential antidotes should be assessed based on their ability to prevent seizure, reduce neuronal damage, and promote survival, as well as preserve quality of life by preventing long-term behavioral abnormalities and cognitive deficiencies.

Present Study

Soman-induced SE is known to produce extensive damage in brain regions (i.e., amygdala, hippocampus, piriform cortex, and thalamus) that are essential to avoidance learning. As such, this study examined whether protection of these brain regions via A1AR agonist treatment would prevent observable cognitive and behavioral deficits.

A two-way active avoidance task was used to assess associative learning and memory retention following exposure. The shuttle box active avoidance test is a high-throughput, automated behavioral assessment. Cognitively intact animals learn to associate warning cues with an impending aversive stimuli (AS) and, ultimately, avoid the AS by employing a shuttle response. An increase in the number of avoidance responses over time is indicative of typical learning and memory processes in rodents

(Quillfeldt, 2016). A1AR agonist compounds were evaluated for their ability to prevent insufficient learning and memory processes following soman exposure. Superior avoidance learning performance was expected from the A1AR agonist-treated animals as compared to their controls treated with saline (vehicle).

Methods

Subjects

Male Sprague-Dawley rats weighing 250 – 350 grams were purchased from Charles River Laboratories (Kingston, NY). Animals were housed in individual cages in temperature (21 ± 2°C) and humidity (50 ± 20%) controlled quarters that were maintained on a 12-hour light-dark cycle (with lights on at 0600 hours). Laboratory chow and tap water were available *ad libitum* whenever animals were in their home cages.

Experiment

Approximately one week before experimentation, animals were anesthetized with isoflurane and surgically fitted with an acrylic headpiece used to measure cortical electroencephalographic (EEG) activity. At that time, a subdermal body temperature transponder (BioMedic Data Systems, Inc., Seaford, DE) was also inserted. Before waking, animals were given an injection of the nonsteroidal anti-inflammatory drug meloxicam (1 mg/kg), as well as the narcotic buprenorphine (0.60 mg/kg) to prevent discomfort.

Animals were separated into eight treatment groups of 12 as detailed in Table 1. On the day of the study, animals were transferred to experimental cages where at least 30 minutes of baseline EEG activity were recorded using CDE 1902 amplifiers and Spike2

software (Cambridge Electronic Design, Ltd., Cambridge, UK). Baseline body weight, temperature and heart rate were also recorded for each animal at that time.

Table 1.

Treatment Groups

Group	Pre-treatment	Exposure	Treatment	*N*
1	HI-6	Saline	Saline + AMN	12
2	HI-6	Saline	CPA + AMN	12
3	HI-6	Saline	CCPA + AMN	12
4	HI-6	Saline	cdENBA + AMN	12
5	HI-6	Soman (1.2xLD_{50})	Saline + AMN	12
6	HI-6	Soman (1.2xLD_{50})	CPA + AMN	12
7	HI-6	Soman (1.2xLD_{50})	CCPA + AMN	12
8	HI-6	Soman (1.2xLD_{50})	cdENBA + AMN	12

Following baseline recording, animals were pre-treated with an intraperitoneal (IP) injection of HI-6 (125 mg/kg) to combat peripheral symptoms. Thirty minutes later, they were challenged with subcutaneously (SC) administered saline or a seizure-inducing dose of soman (1.2 x LD_{50}). After one minute, animals were treated with intramuscular (IM) injections of atropine methylnitrate (AMN; 2 mg/kg) and saline (0.5 ml/kg, IP), CPA (60 mg/kg, IP), CCPA (36 mg/kg, IP) or cdENBA (62 mg/kg, IP) at the previously established minimum effective dose (Thomas et al., 2018).

Following saline or soman exposure, animals were observed continuously for five hours. In addition to EEG activity, heart rate and body temperature, each animal's righting reflex *(See Table 2*) was monitored. All signs were recorded again at 24 hours post-exposure and once daily for the duration of the experiment (up to 14 days after exposure).

Animals underwent a series of three behavioral testing sessions with a shuttle box avoidance assay at three (session 1), seven (session 2) and fourteen days (session 3) post-

exposure. Following the final testing session on day fourteen, animals were transcardially perfused with saline solution and fixed with 10% formalin. Hematoxylin and eosin (H&E) stained sections of key brain regions were then evaluated and scored by a trained pathologist using established methodology (McDonough et al., 1995; See Table 3).

Table 2.

Righting Reflex Observation Score Sheet

	Observation	Score
Righting Reflex	Normal (Immediate righting)	0
	Impaired (<1 second)	1
	Impaired (>1 second)	2
	Non-responsive	3

Behavioral Procedure

Shuttle Box Assessment. Animals were subjected to a series of three shuttle box testing sessions at three (session 1), seven (session 2) and fourteen days (session 3) after soman exposure. All animals were given a five-minute acclimation period to the shuttle box (Gemini System, San Diego Instruments, Inc., San Diego, CA, USA) at the start of each session. Both compartments were dark for the duration of the acclimation. Each compartment measured 24 cm x 20 cm x 20 cm and was equipped with 16 infrared sensors to detect animal location. Both compartments also encompassed one speaker, one overhead light and one cue light positioned 12.5 cm above the grid floor. All grids were pre-tested to confirm stable output intensity.

Immediately following acclimation, animals were presented with the warning stimulus (WS) which consisted of the simultaneous activation of the house light, cue light, and 75 dB tone in the compartment that housed the animal. Five seconds later, animals experienced the AS, a 1.2 mA scrambled shock, for up to 20 seconds. Leaving

the compartment terminated both the WS and AS and began a 15- to 25-second inter-trial interval (ITI). Shuttle responses were divided into three categories: An avoidance response (leaving the compartment before the AS based on the cues of the WS), an escape response (leaving the compartment after experiencing the AS), or no response (unsuccessfully terminating the AS; *see Figure 1*). Each session included 50 trials. Testing was terminated after 10 consecutive trials with no response or at a maximum of 45 minutes.

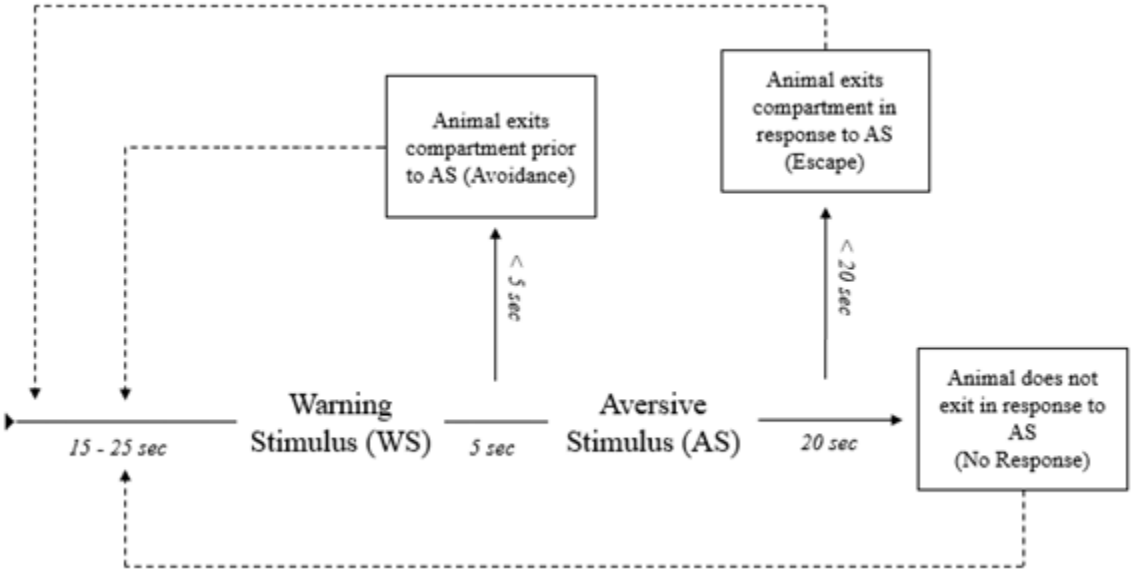

Figure 1.

Shuttle Box Assessment Paradigm.

[1] The 15- to 25-second inter-trial interval is initially preceded by a 5-minute acclimation period.

Data Analysis

Physiological Effects. Descriptive analyses of the physiological variables (i.e., heart rate, temperature and body weight) in the sample indicated no significant skew or

deviation from normality. Therefore, changes in heart rate, temperature and body weight were compared across A1AR agonist-treated groups with a one-way analysis of variance (ANOVA). Following significant main effects, Bonferroni comparisons were made between groups. Rates of seizure prevention were compared using the Fisher's exact test.

Behavior. Due to a lack of homogeneity of variance within the sample, non-parametric statistics were implemented to represent all behavioral output. The number of avoidance responses demonstrated and the median amount of time the AS was experienced (up to a maximum of 20 seconds) prior to escape were used as measures of each animal's performance across all trials for each of the three sessions. Median scores were then calculated for each of the eight experimental groups. Mann-Whitney *U* tests were used to compare the number of avoidance responses employed, and the duration of time the AS was experienced by each A1AR agonist-treated group against the respective saline-treated control group, as well as to assess within group differences in the number of avoidance responses demonstrated from session one to session three. The Kruskal-Wallis *H* test was used to compare the average AS time and the number of avoidance responses among A1AR agonist-treated groups. Following significant main effects, paired comparisons were made using Mann-Whitney *U* statistics. Animals that died within 3 days of soman exposure did not undergo the initial shuttle box assessment and were, therefore, excluded from behavioral analyses.

Neuropathology and Lethality. Regions associated with neurological deficits following soman exposure (i.e., amygdala, dorsal and ventral hippocampus, piriform cortex or thalamus) were analyzed and scored on a scale of 0 – 4 (0 = no damage, 4 =

severe damage). Magnitude of total brain damage was assessed by summing the neuropathology scores of the five regions (0 = no damage, 20 = severe damage). Lethality was compared between the control and individual treatment groups using the Fisher's exact test.

Statistical significance was defined as $p < .05$ for all tests.

Table 3.

Neuropathology Severity Score Sheet

Lesion Observation	Score
No lesion	0
Minimal (1-10% tissue involvement)	1
Mild (11-25% tissue involvement)	2
Moderate (26-45% tissue involvement)	3
Severe (>45% tissue involvement)	4

Results

I. Saline Exposure.

Sedation. In saline-exposed animals, righting reflex was affected by the administration of all three A1AR-agonist compounds, indicating rapid sedative effects consistent with the results of previous studies (Thomas et al., 2018; *See Figure 2*). While sedation following cdENBA treatment occurred the fastest (median score > 2 within 4 minutes of administration), animals also regained righting ability (median score of 2 at 3.5 hours post-administration) the fastest (*See Figure 3*) among the A1AR-treated groups. By 5 hours post-administration, all animals treated with cdENBA demonstrated normal baseline scores for righting ability. Animals treated with CPA or CCPA did not display a righting reflex (median score > 2) until 24 hours later. Normal righting ability was demonstrated 48 hours after treatment.

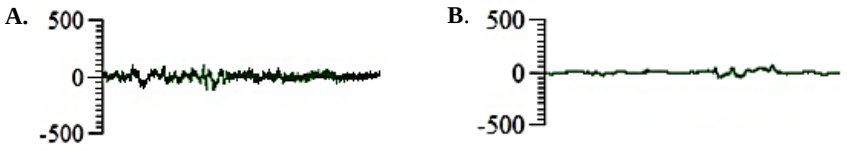

Figure 2. *Visual Differences in EEG Activity.* Differences between (A) typical baseline EEG recording and (B) the isoelectric state induced by A1AR agonist compounds indicating deep sedation.

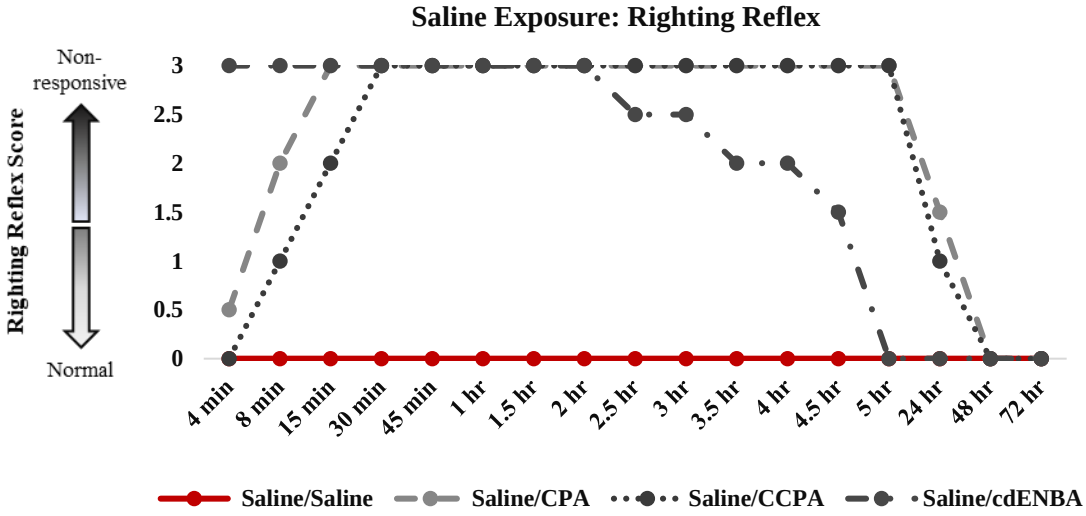

Figure 3. *Median Righting Reflex Score among Rats Challenged with Saline*

Lethality. Among saline-exposed groups, two animals treated with CPA (16.67%, $N = 12$) and one animal treated with CCPA (8.33%, $N = 12$) died prior to the end of the study (*See Table 4*). Both saline/CPA-treated animals that did not survive the duration of the study were found dead 3 days post-exposure (one prior to and one following the first session of shuttle box testing). The saline/CCPA-treated animal was found dead 24 hours post-exposure. All animals in the saline/saline and saline/cdENBA groups survived the duration of the study.

Table 4.

14-Day Survival Rates among Rats Challenged with Saline

Group	Treatment	*N*	% Survival
1	Saline	12	12 (100%)
2	CPA	12	10 (83.30%)
3	CCPA	12	11 (91.6%)
4	cdENBA	12	12 (100%)

Physiological Effects. As expected, animals treated with A1AR agonists experienced decreases in heart rate and body temperature. One-way ANOVA indicated that heart rate recovery relative to baseline did not differ significantly among saline/CPA (47.22% ± 0.05, $N = 12$), saline/CCPA (64.27% ± 0.10, $N = 7$), and saline/cdENBA (64.25% ± 0.05, $N = 11$) groups 24 hours after delivery of treatment ($p = 0.088$; *See Figure 4 & Table 5*). Significant differences in body temperature between saline/CPA (81.23% ± 0.03) and saline/cdENBA (97.10% + 0.01, $p < .001$), as well as saline/CPA and saline/CCPA (92.79% ± 0.03, p = .009) groups at 24 hours resolved by 48 hours, when no significant difference among the three groups was found ($p = .244$; *See Figure 5 & Table 6*).

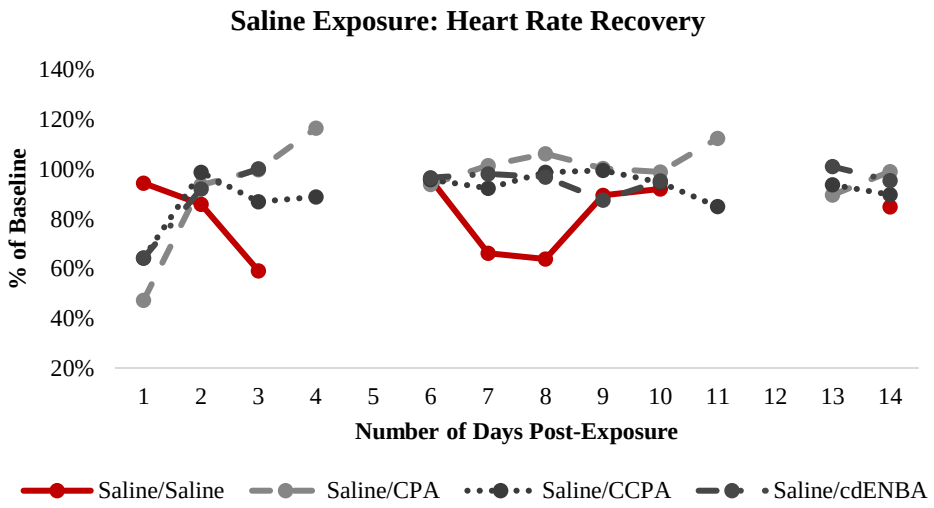

Figure 4.

Average Heart Rate Recovery Relative to Baseline among Rats Challenged with Saline

Table 5.

Heart Rate Recovery (% of Baseline[1]) among Rats Challenged with Saline

Day	Saline	CPA	CCPA	cdENBA
	Mean, SEM	Mean, SEM	Mean, SEM	Mean, SEM
Exp Min	89.29% ± 0.06	26.84% ± 0.05	28.10% ± 0.04	32.27% ± 0.03
1	94.36% ± 0.05	47.22% ± 0.05	64.27% ± 0.10	64.25% ± 0.05
2	85.84% ± 0.03	93.54% ± 0.06	98.74% ± 0.07	92.06% ± 0.06
3	59.09% ± 0.16	99.82% ± 0.11	86.87% ± 0.09	100.20% ± 0.04
4		116.46% ± 0.09	88.78% ± 0.08	
5				
6	95.97% ± 0.05	93.90% ± 0.02	95.77% ± 0.03	96.48% ± 0.04
7	66.20% ± 0.18	101.49% ± 0.05	92.28% ± 0.03	98.03% ± 0.04
8	63.81% ± 0.17	106.16% ± 0.05	98.68% ± 0.03	96.76% ± 0.04
9	89.43% ± 0.02	100.23% ± 0.06	99.47% ± 0.05	87.51% ± 0.05
10	92.06% ± 0.02	98.85% ± 0.06	94.55% ± 0.03	95.22% ± 0.04
11		112.40% ± 0.05	85.01% ± 0.07	
12				
13		89.53% ± 0.04	93.73% ± 0.04	101.00% ± 0.04
14	84.83% ± 0.04	99.04% ± 0.07	89.76% ± 0.04	95.39% ± 0.06

[1] Baseline heart rates were taken the day of the experiment, prior to exposure or treatment.

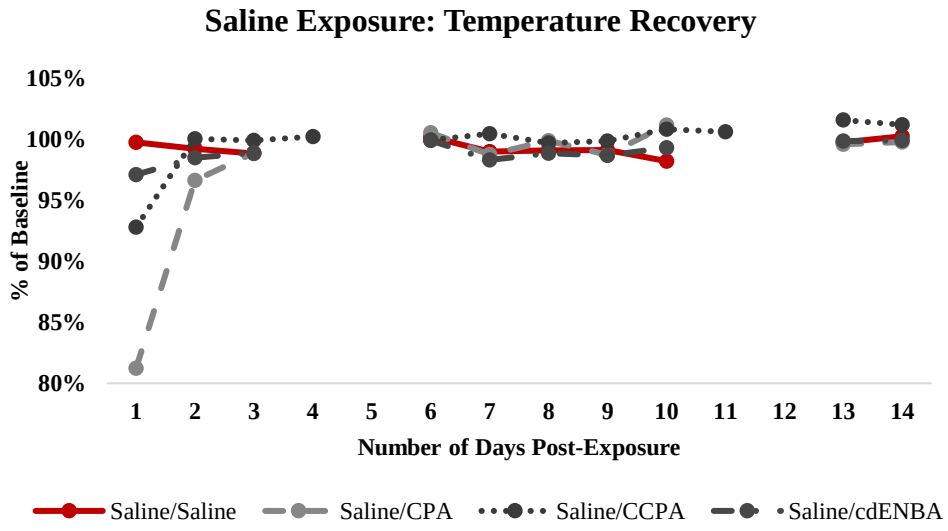

Figure 5.

Average Temperature Recovery Relative to Baseline among Rats Challenged with Saline

Table 6.

Temperature Recovery (% of Baseline) among Rats Challenged with Saline

Day	Saline	CPA	CCPA	cdENBA
	Mean, SEM	Mean, SEM	Mean, SEM	Mean, SEM
Exp Min	95.61% ± 0.01	85.66% ± 0.03	86.89% ± 0.03	86.20% ± 0.02
1	99.74% ± 0.00	81.23% ± 0.03	92.79% ± 0.03	97.10% ± 0.01
2	99.21% ± 0.00	96.63% ± 0.02	100.04% ± 0.00	98.48% ± 0.00
3	98.83% ± 0.00	98.87% ± 0.01	99.91% ± 0.00	98.87% ± 0.00
4			100.21% ± 0.01	
5				
6	100.20% ± 0.00	100.53% ± 0.00	99.93% ± 0.00	99.90% ± 0.00
7	98.99% ± 0.00	98.76% ± 0.00	100.46% ± 0.00	98.29% ± 0.00
8	99.08% ± 0.00	99.87% ± 0.00	99.70% ± 0.01	98.85% ± 0.00
9	99.10% ± 0.00	98.73% ± 0.01	99.86% ± 0.00	98.67% ± 0.00
10	98.20% ± 0.00	101.17% ± 0.01	100.81% ± 0.00	99.29% ± 0.00
11			100.61% ± 0.01	
12				
13	99.74% ± 0.00	99.58% ± 0.01	101.57% ± 0.00	99.85% ± 0.00
14	100.24% ± 0.00	99.77% ± 0.00	101.19% ± 0.00	99.89% ± 0.00

Body weight was reduced relative to baseline at 24 hours among all four saline-exposed groups (*See Figure 6 & Table 7*). Saline/CPA was the only group that did not exceed baseline body weight seven days after exposure (96.57% ± 1.42). While all four groups averaged body weights greater than their baseline weight at the conclusion of the study (day 14), rats administered CPA (105.24% ± 1.83) gained less weight than rats administered cdENBA (109.12% + 2.07), and significantly less than rats administered CCPA (112.34% ± 1.15, $p = .027$). There was no significant difference between cdENBA- and CCPA-treated animals.

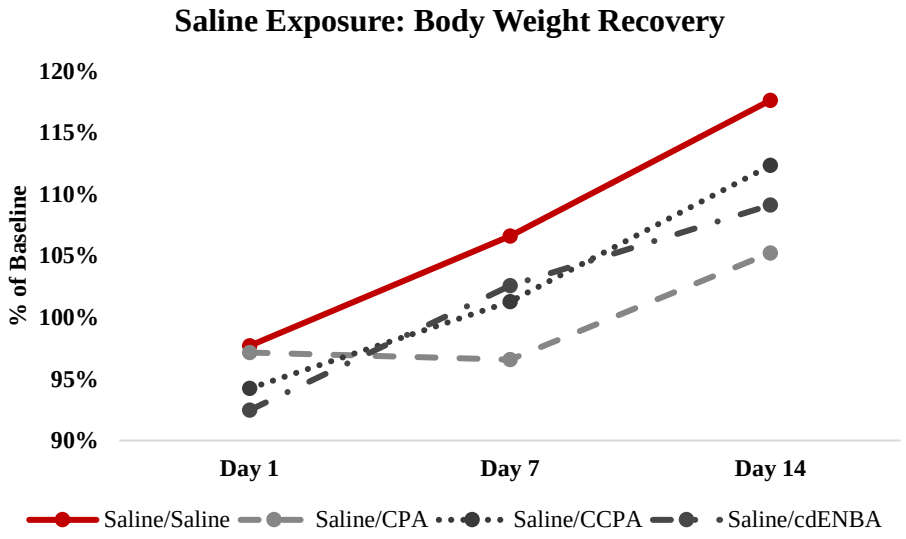

Figure 6.

Average Body Weight Recovery Relative to Baseline among Rats Challenged with Saline

Table 7.

Body Weight Recovery (% of Baseline) among Rats Challenged with Saline

Day	Saline	CPA	CCPA	cdENBA
	Mean, SEM	Mean, SEM	Mean, SEM	Mean, SEM
1	97.69% ± 0.30	97.14% ± 0.90	94.23% ± 1.12	92.46% ± 0.56
7	106.60% ± 0.73	96.57% ± 1.42	101.27% ± 0.98	102.59% ± 0.83
14	117.63% ± 1.05	105.24% ± 1.83	112.34% ± 1.15	109.12% ± 2.07

Behavior. A1AR agonist compounds were administered to animals challenged with saline (experimental groups 2-4) to assess each drug's independent effect on avoidance learning. One additional group of rats (Group 1) was challenged as well as treated with saline to establish a baseline for comparison. Figure 7 represents the number of avoidance and escape responses for each group across sessions. No statistically significant differences in number of avoidance responses were found between the saline-treated control group and any of the A1AR agonist-treated groups, during any session (*See Figure 8 & Table 8*). A Kruskal-Wallis *H* test revealed no significant differences among the three A1AR agonist-treated groups in sessions one ($p = .575$), two ($p = .278$)

or three (p = .562). In addition to the saline-treated control group, Mann-Whitney U tests indicated all three A1AR agonist-treated groups (i.e., saline/CPA, saline/CCPA, and saline/cdENBA) demonstrated significant increases in the number of avoidance responses employed during session three, as compared to session one (p = .003, p < .001, p = .001, respectively; *See Figure 8*).

When escape responses were employed, the amount of time each animal experienced the AS prior to exiting the compartment trended downward across sessions for all four groups (*See Figure 9 & Table 9*). Saline/CPA-treated animals demonstrated the only incidence of significantly greater median AS time (112.30ms) than the control group (67.34ms, p = .006). This difference was, however, only present during the first session. Significant differences were found among the three A1AR agonist-treated groups during session one (p = .003). Pairwise comparisons indicated differences between CPA and CCPA (p = .004), and CPA and cdENBA (p = .022). No significant differences among the A1AR agonist-treated groups were identified in sessions two (p = .236) or three (p = .263).

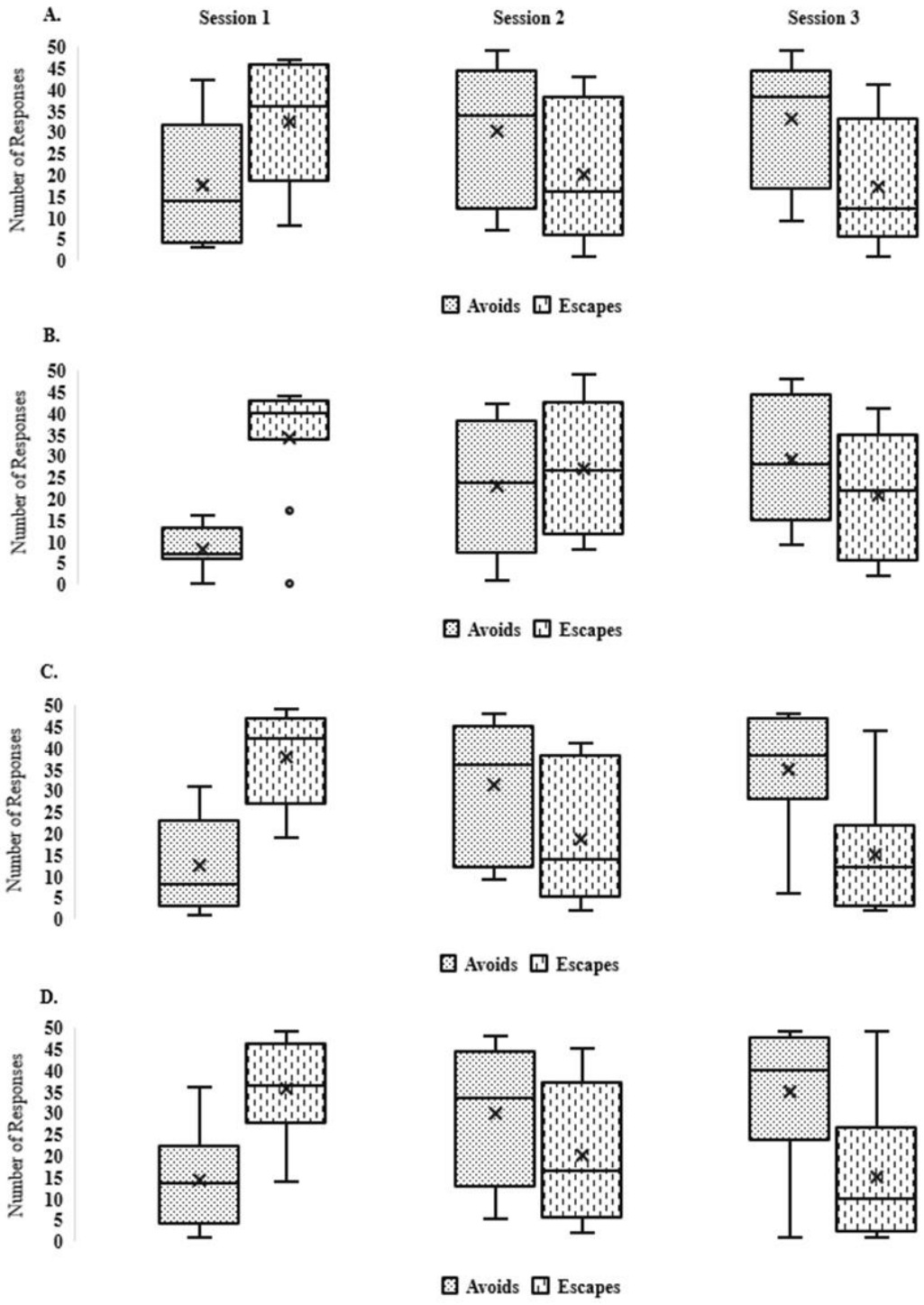

Figure 7.

Shuttle Responses across Sessions among Rats Challenged with Saline

Number of avoidance and escape responses for saline/saline (A), saline/CPA (B), saline/CCPA (C), and saline/cdENBA (D) groups across all three testing sessions.

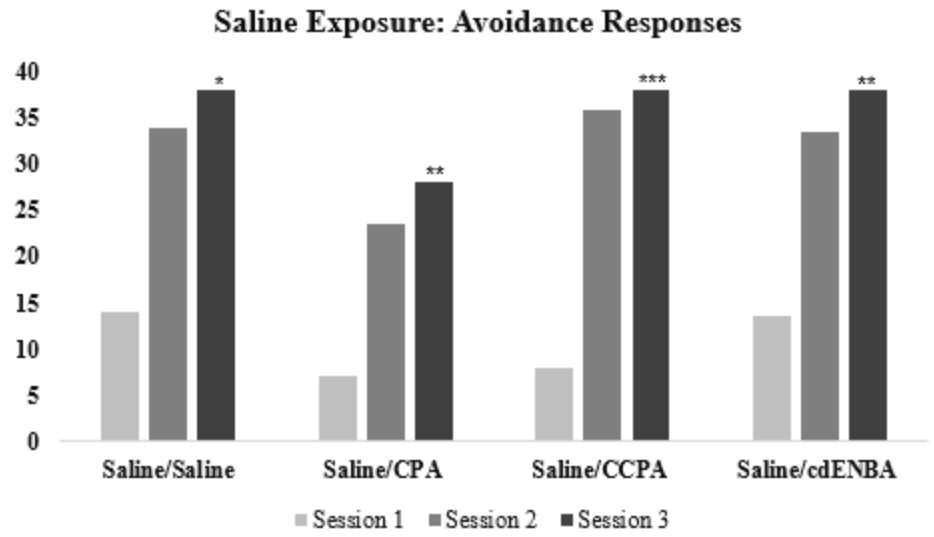

Figure 8.

Median Number of Avoidance Responses among Rats Challenged with Saline

Statistically significant within group differences between session 3 and session 1 are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 8.

Avoidance Responses among Rats Challenged with Saline

Group	Treatment	*N*	Session	Median # Avoids	Range
1	Saline	12	1	14.00	(3.00, 42.00)
		12	2	34.00	(7.00, 49.00)
		12	3	38.00*	(9.00, 49.00)
2	CPA	11	1	7.00	(0.00, 16.00)
		10	2	23.50	(1.00, 42.00)
		10	3	36.00***	(9.00, 48.00)
3	CCPA	11	1	8.00	(1.00, 31.00)
		11	2	36.00	(9.00, 48.00)
		11	3	38.00***	(6.00, 48.00)
4	cdENBA	12	1	13.50	(1.00, 36.00)
		12	2	33.50	(5.00, 48.00)
		12	3	40.00**	(1.00, 49.00)

Statistically significant within group differences between session one and session three are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

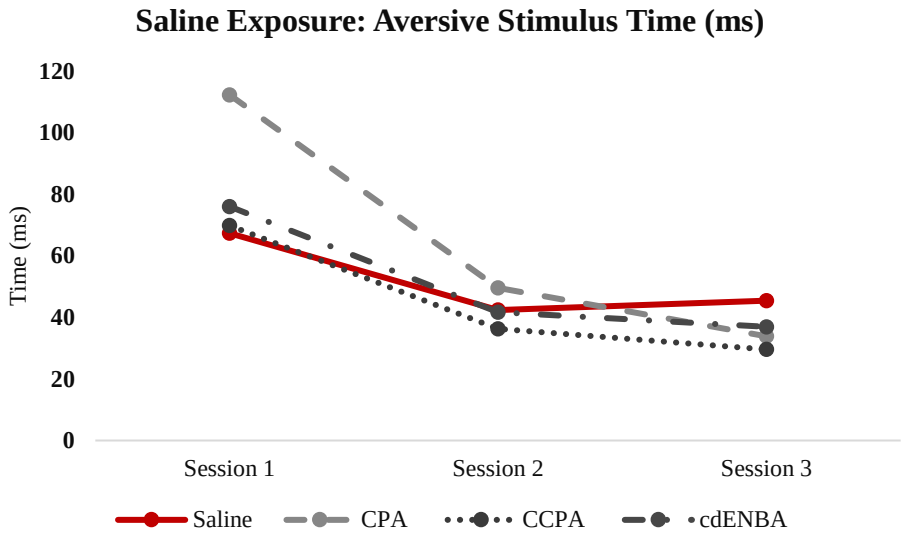

Figure 9.

Median Aversive Stimulus Time among Rats Challenged with Saline

Table 9.

Aversive Stimulus Time among Rats Challenged with Saline

Group	Treatment	*N*	Session	Median AS Time (ms)	Range
1	Saline	12	1	67.34	(33.56, 122.35)
		12	2	42.38	(8.00, 130.47)
		12	3	45.43	(17.33, 103.32)
2	CPA	11	1	112.30**	(72.49, 1500.00)
		10	2	49.56	(21.85, 217.00)
		10	3	33.86	(26.39, 185.27)
3	CCPA	11	1	69.84	(40.37, 103.60)
		11	2	36.31	(15.50, 87.27)
		11	3	29.58	(14.67, 90.91)
4	cdENBA	12	1	76.03	(36.57, 137.07)
		12	2	41.70	(20.00, 94.42)
		12	3	36.94	(19.78, 85.00)

Statistically significant differences when comparing each A1AR agonist-treated group against the Saline/Saline control for each respective session are indicated by $^{**}p < 0.01$.

Neuropathology. Although neuropathology could not be observed or scored when animals did not survive the duration of the study, no neuropathology was observed

in the saline/saline control group or any of the saline/A1AR agonist-treated groups (all regions of all brains evaluated received scores of 0).

II. Soman Exposure.

Seizure prevention. EEG was used to detect the onset of seizure among soman-exposed animals (Table 1; groups 5-8). Presence of EEG seizure was determined by visual assessment of changes in amplitude and frequency (i.e., transition to severe spiking activity with high amplitude [>±1000 μV], high frequency) from baseline (i.e., low amplitude [<±100 μV], high frequency; *See Figure 10*). Among soman-exposed, saline-treated (soman/saline) rats, the rate of seizure onset was 100% (12/12). Seizure was prevented in 91.67% (11/12) of soman/CPA, 83.33% (10/12) of soman/CCPA and 75.00% (9/12) of soman/cdENBA groups (*See Table 10*). While saline-treated animals demonstrated abnormal EEG spiking activity throughout the duration of the experiment, no A1AR agonist-treated animal that was protected from SE during the initial experiment day exhibited seizure during recording on any of the subsequent days.

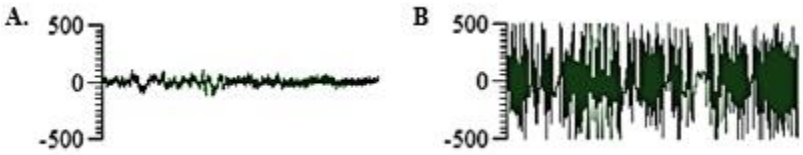

Figure 10.

Visual Differences in EEG Activity. Differences between (A) typical baseline EEG recording and (B) onset of *SE*.

Table 10.

Seizure Prevention Rates among Rats Challenged with Soman

Group	Treatment	*N*	Seizure Prevention
5	Saline	12	0 (0.00%)
6	CPA	12	11 (91.67%)***
7	CCPA	12	10 (83.33%)***
8	cdENBA	12	9 (75.00%)***

Statistically significant differences between soman/saline control and A1AR-agonist treated groups are indicated by ***$p < 0.001$.

Sedation. Righting reflex was affected by each of the three A1AR agonist compounds during the experiment (*See Figure 11*). Animals in the soman/saline group demonstrated a prolonged, non-responsive state following exposure due to SE and other toxic signs. Animals in all three A1AR agonist-treated groups regained righting ability (median score < 2) before animals in the soman/saline control group. Of the three A1AR agonist-treated groups, CPA-treated animals took the longest to return to typical righting behavior (median score of 1 at 24 hours after soman exposure).

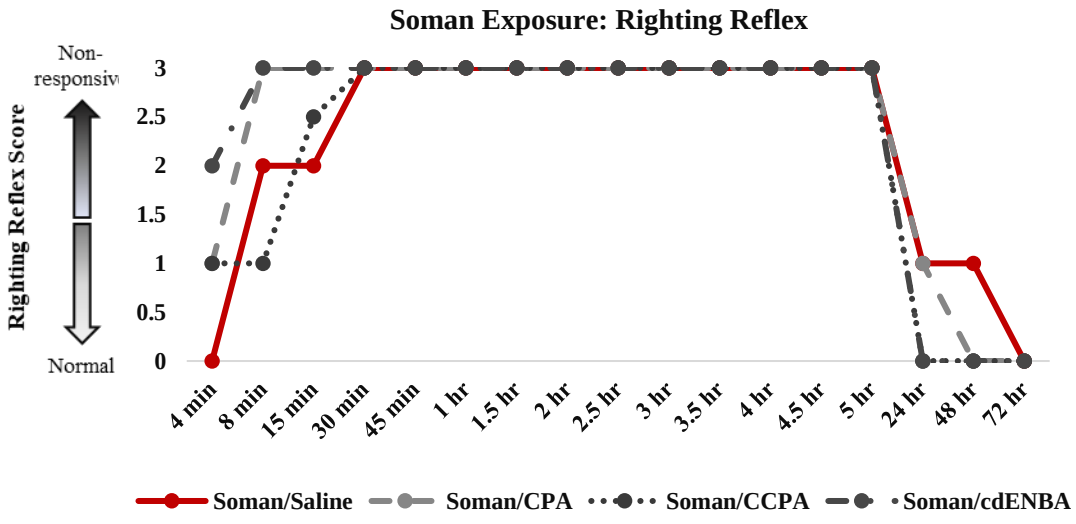

Figure 11. *Median Righting Reflex Score among Rats Challenged with Soman*

Survival. Among 12 soman/saline-treated animals, 58.33% survived the entire duration of the experiment. The average survival time of the 5 non-survivors was 4.16 hours (± 0.78). Four of the animals died prior to the end of the initial experiment day, and one was found dead at 24 hours post-exposure. Of the A1AR agonist-treated groups, survival was the highest among animals in the soman/CPA group (83.33%; *See Figure 12 & Table 11).* Time of death for both non-surviving CPA-treated animals was reported as 7.00 hours from exposure, indicating survival through the end of the first experiment day, and time of death sometime before the next morning. The soman/CCPA group demonstrated 58.33% survival. The average length of survival among non-surviving animals in the CCPA-treated group was 11.21 hours (± 4.53). Two of the non-surviving animals died the day of the initial experiment, and 3 additional animals died at some point during night 1 (prior to 24-hour observation time). Survival was the lowest among the cdENBA-treated animals (36.36%). Mean survival time among non-survivors was also lowest for the soman/cdENBA group (3.84 hours ± 1.06). Six cdENBA-treated animals died during the initial experiment day, while one was found dead at 24 hours post-exposure, and one additional animal died 2 days after exposure. One animal from the cdENBA-treated group was removed from the study two days after exposure due to a dislocated headpiece. This animal may have lived for the duration of the experiment. Overall, no deaths occurred after day 3 (i.e., 72 hours after soman exposure).

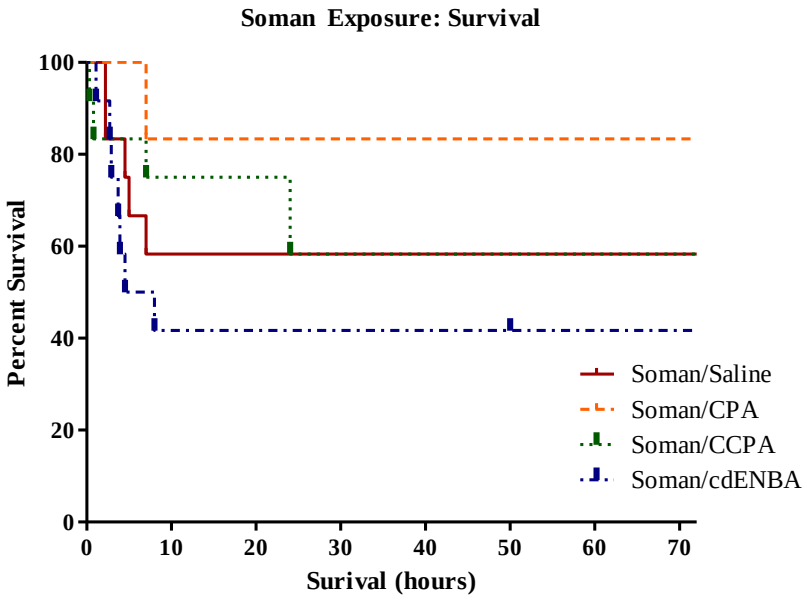

Figure 12.

72-Hour Survival among Rats Challenged with Soman

Note: All animals that survived to 72 hours also survived the duration of the study (14 days). Animals from the soman/saline group survived 197.73 hours (± 49.30) on average, as compared to the soman/CPA (281.17 ± 37.0), soman/CCPA (200.00 ± 167.40), and soman/cdENBA groups (124.62 ± 48.40).

Table 11.

14-Day Survival among Rats Challenged with Soman

Treatment	*N*	% Survival
Saline	12	58.33% (7/12)
CPA	12	83.33% (10/12)
CCPA	12	58.33% (7/12)
cdENBA	11	36.36%[1] (4/11)

[1]One animal was removed from the study due to a dislocated headpiece 2 days after exposure.

Physiological Effects. Average heart rates relative to baseline among soman-exposed, A1AR agonist-treated groups were greatly reduced at 24 hours post-exposure, compared to the soman/saline control group (98.39% of baseline; *Figure 13 & Table 12*). One-way ANOVA indicated no significant differences in heart rate recovery (p = .088) among groups treated with CPA (61.16% ± 0.08), CCPA (67.08% ± 0.08) or cdENBA (58.85% ± 0.06) at that time point. By 48 hours post-exposure, average heart rates had increased among soman/CPA (97.80% ± 0.05), soman/CCPA (103.46% + 0.06), and soman/cdENBA (105.99% ± 0.03) groups. While temperature was most drastically reduced by 24 hours post-exposure in the soman/CPA group (87.78% ± 0.02), it rebounded by 48 hours (98.62% ± 0.02; *see Figure 14 & Table 13*). At 72 hours post-exposure, average temperatures among soman/CPA (99.62% ± 0.01), soman/CCPA (99.79% ± 0.00), soman/cdENBA (100.00% ± 0.00) groups were higher relative to baseline than in animals in the soman/saline control group (97.18% ± 0.01).

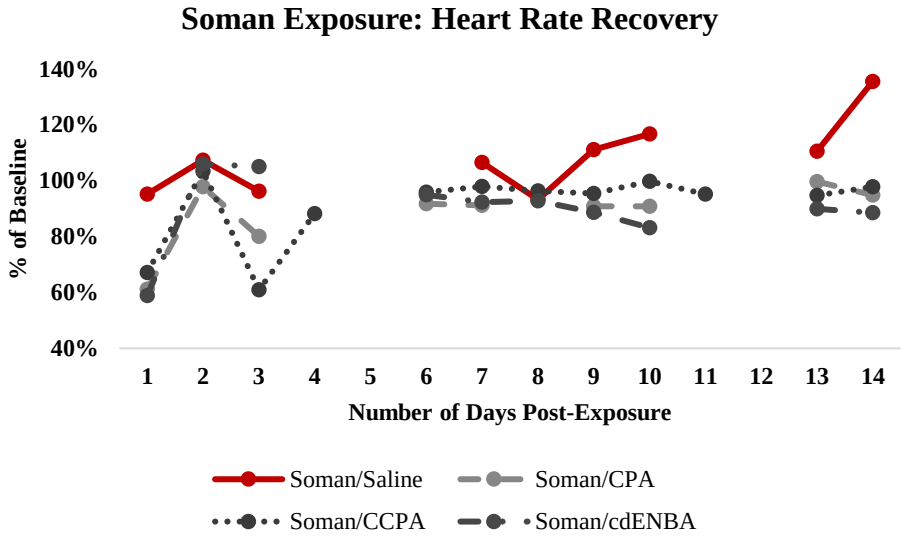

Figure 13.

Average Heart Rate Recovery Relative to Baseline among Rats Challenged with Soman

Table 12.

Heart Rate Recovery (% of Baseline) among Rats Challenged with Soman

Day	Saline	CPA	CCPA	cdENBA
	Mean, SEM	Mean, SEM	Mean, SEM	Mean, SEM
Exp Min	38.81% ± 0.11	29.53% ± 0.04	30.35% ± 0.09	38.19% ± 0.11
1	95.15% ± 0.10	61.16% ± 0.08	67.08% ± 0.08	58.85% ± 0.06
2	107.25% ± 0.12	97.80% ± 0.05	103.46% ± 0.06	105.99% ± 0.03
3	96.19% ± 0.13	80.11% ± 0.12	60.90% ± 0.21	105.01% ± 0.12
4			88.14% ± 0.00	
5				
6		91.72% ± 0.04	95.82% ± 0.03	94.93% ± 0.02
7	106.54% ± 0.15	91.14% ± 0.03	97.86% ± 0.03	92.26% ± 0.04
8	93.23% ± 0.07		96.35% ± 0.04	92.81% ± 0.10
9	111.11% ± 0.13	90.79% ± 0.04	95.45% ± 0.05	88.68% ± 0.06
10	116.75% ± 0.16	90.847% ± 0.03	99.76% ± 0.04	83.16% ± 0.02
11			95.15% ± 0.00	
12				
13	110.47% ± 0.11	99.67% ± 0.04	94.67% ± 0.06	89.93% ± 0.04
14	135.49% ± 0.22	94.83% ± 0.05	97.79% ± 0.05	88.51% ± 0.03

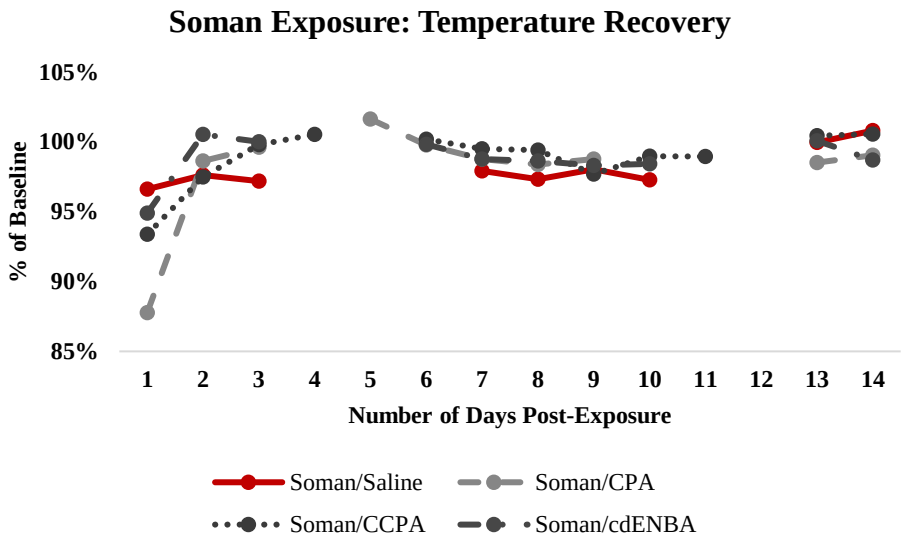

Figure 14.

Average Temperature Recovery Relative to Baseline among Rats Challenged with Soman

Table 13.

Temperature Recovery (% of Baseline) among Rats Challenged with Soman

Day	Saline	CPA	CCPA	cdENBA
	Mean, SEM	Mean, SEM	Mean, SEM	Mean, SEM
Exp Min	96.12% ± 0.03	83.49% ± 0.05	89.85% ± 0.06	91.63% ± 0.05
1	96.60% ± 0.01	87.78% ± 0.02	93.37% ± 0.02	94.89% ± 0.01
2	97.62% ± 0.01	98.62% ± 0.02	97.49% ± 0.01	100.52% ± 0.00
3	97.18% ± 0.01	99.62% ± 0.01	99.79% ± 0.00	100.00% ± 0.00
4			100.53% ± 0.00	
5				
6		101.62% ± 0.00	100.18% ± 0.00	99.81% ± 0.00
7	97.90% ± 0.01	99.77% ± 0.00	99.48% ± 0.00	98.76% ± 0.01
8	97.31% ± 0.01	99.77% ± 0.00	99.41% ± 0.01	98.62% ± 0.01
9	98.02% ± 0.01	98.37% ± 0.00	97.68% ± 0.01	98.29% ± 0.01
10	97.27% ± 0.01	98.75% ± 0.00	98.961% ± 0.01	98.42% ± 0.00
11			98.95% ± 0.00	
12				
13	99.96% ± 0.01	99.51% ± 0.01	100.44% ± 0.01	100.07% ± 0.01
14	100.79% ± 0.00	99.04% ± 0.01	100.54% ± 0.01	98.69% ± 0.01

Animals from the soman/saline group were the slowest to recover their initial body weight and gain additional weight following exposure (*See Figure 15 & Table 14*). At 24 hours, their body weights were reduced to 89.23% (± 0.64) of baseline, compared to the soman/CPA (91.14% ± 1.51), soman/CCPA (93.26% ± 0.96), and soman/cdENBA groups (90.85% + 0.96). Animals from the soman/saline group represented the lowest weight gain relative to baseline of the four groups seven days post-exposure. At the end of the study, animals from the soman/saline group had exceeded their average baseline weights (103.32% ± 5.38) but still gained less weight than the soman/CPA (108.93% ± 2.20), soman/CCPA (110.34% ± 2.23) or soman/cdENBA (109.60% + 0.76) groups at that time.

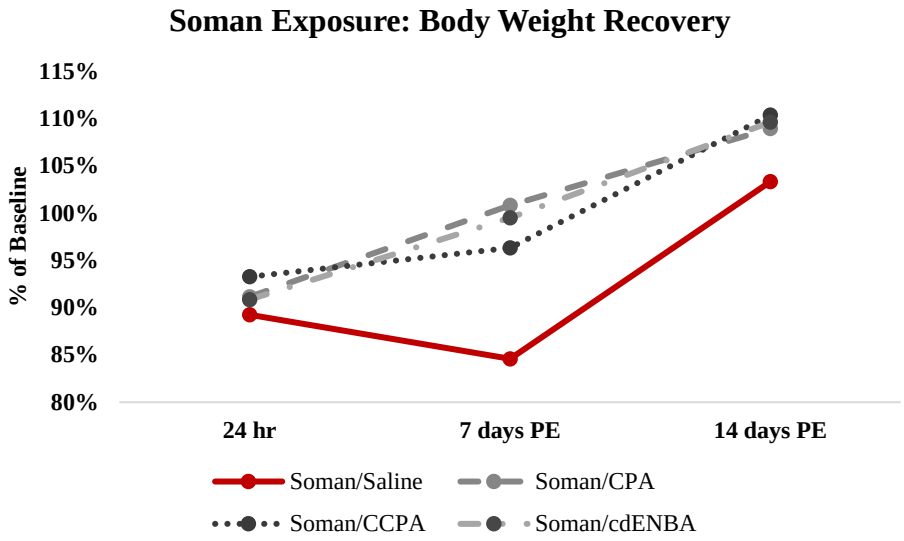

Figure 15.

Average Body Weight Recovery Relative to Baseline among Rats Challenged with Soman

Table 14.

Body Weight Recovery (% of Baseline) among Rats Challenged with Soman

Day	Saline	CPA	CCPA	cdENBA
	Mean, SEM	Mean, SEM	Mean, SEM	Mean, SEM
1	89.23% ± 0.64	91.14% ± 1.51	93.26% ± 0.96	90.85% ± 0.96
7	84.59% ± 5.59	100.81% ± 1.83	96.33% ± 2.77	99.50% ± 2.81
14	103.32% ± 5.38	108.93% ± 2.20	110.34% ± 2.23	109.60% ± 0.76

Behavior. Among rats challenged with soman, those treated with saline demonstrated a significantly lower median number of avoidance responses (2.00) during session one than those treated with CPA (11.50, $p = .007$), CCPA (19.00, $p = .001$) or cdENBA (29.00, $p = .006$; *See Figure 16 & Table 15*). During session two, differences in the median number of avoidance responses employed between soman/saline (6.00) and soman/CPA (21.50) approached significance ($p = .055$). Significant differences were found between the soman/saline and soman/cdENBA (31.50, $p = .042$), as well as the soman/saline and soman/CCPA (44.00, $p = .002$), groups. The median number of

avoidance responses in session three still differed significantly between soman/saline (15.00) and soman/CCPA (48.00, $p = .002$) groups. A Kruskal-Wallis test indicated no significant differences in the number of avoidance responses exhibited among soman-exposed, A1AR agonist-treated groups in sessions one ($p = .124$), two ($p = .203$) or three ($p = .198$). The number of avoidance and escape responses during each session across treatment groups is represented in Figure 17.

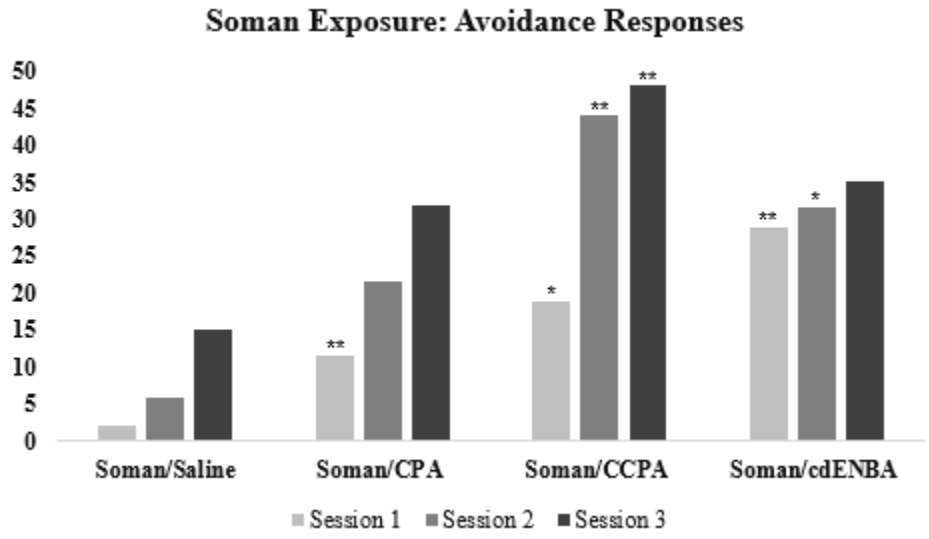

Figure 16.

Median Number of Avoidance Responses among Rats Challenged with Soman

Statistically significant differences between soman/saline and each A1AR agonist-treated group are indicated by $*p < 0.05$ and $**p < 0.01$.

Table 15.

Avoidance Responses among Rats Challenged with Soman

Treatment	*N*	Session	Median # Avoids	Range
Saline	7	1	2.00	0.00, 9.00
	7	2	6.00	1.00, 20.00
	7	3	15.00	2.00, 35.00
CPA	10	1	11.50**	2.00, 39.00
	10	2	21.50	0.00, 44.00
	10	3	32.00	1.00, 50.00
CCPA	7	1	19.00***	9.00, 48.00
	7	2	44.00**	9.00, 50.00
	7	3	48.00**	27.00, 50.00
cdENBA	4	1	29.00**	10.00, 47.00
	4	2	31.50*	6.00, 42.00
	4	3	35.00	2.00, 48.00

Statistically significant differences when comparing each A1AR agonist-treated group against the soman/saline control for each session are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

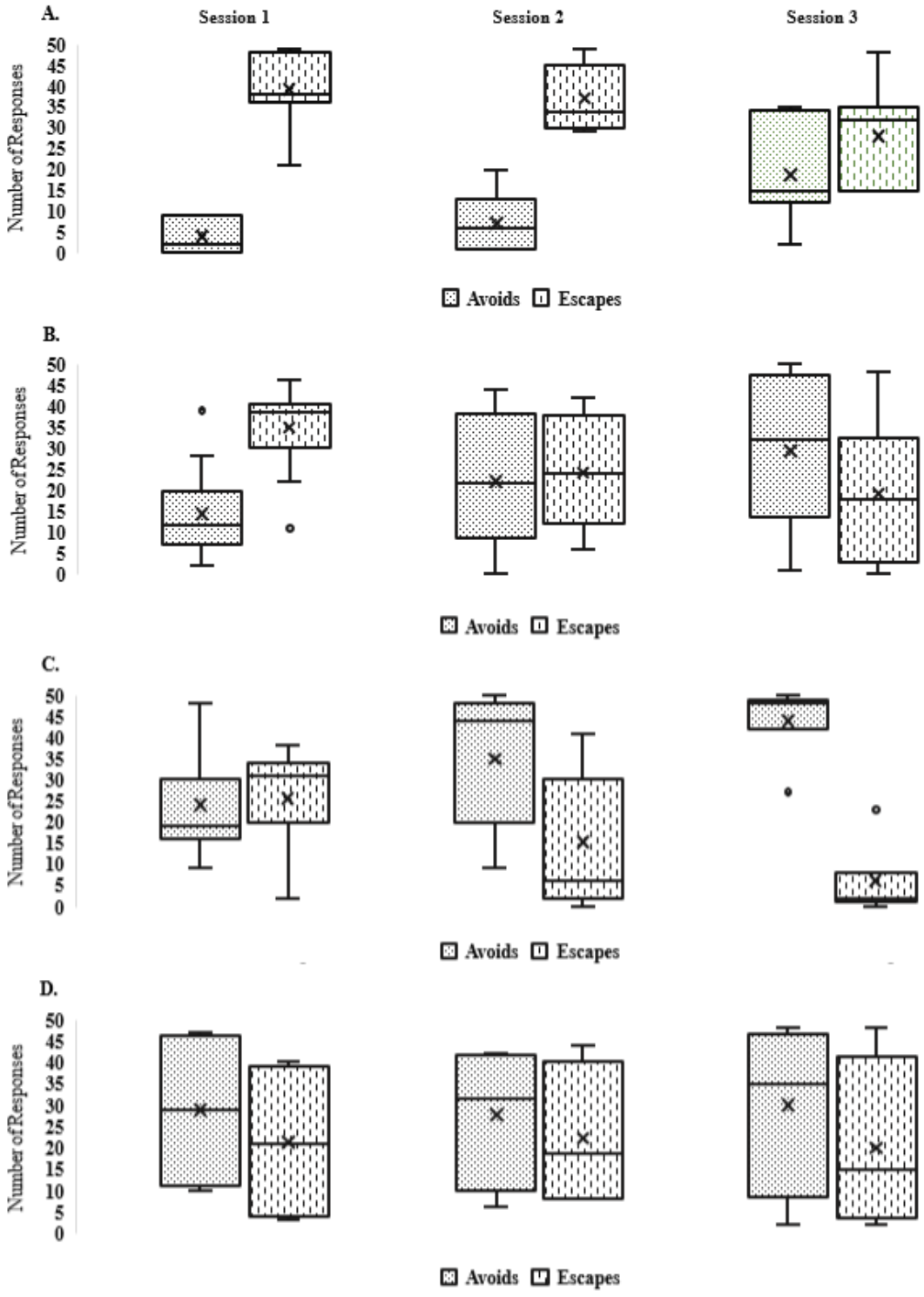

Figure 17.

Shuttle Responses across Sessions among Rats Challenged with Soman

Number of avoidance and escape responses for soman/saline (A), soman/CPA (B), soman/CCPA (C), and soman/cdENBA (D) groups across all three testing sessions.

Median AS times experienced by the soman/saline group during sessions one (337.00ms), two (339.68ms) and three (215.35ms) were significantly greater than those experienced by the soman/CPA (78.70ms, $p = .001$; 53.67ms, $p = .025$; 41.20ms, $p = .003$, respectively), soman/CCPA (56.74ms, $p = .007$; 36.17ms, $p = .001$; 25.00ms, $p = .001$, respectively), and soman/cdENBA groups (41.65ms, $p = .006$; 39.89ms, $p = .006$; 37.41ms, $p = .006$, respectively; *See Figure 18 & Table 16*). A Kruskal-Wallis test indicated that there were no significant differences in AS exposure time among the three A1AR agonist-treated groups in sessions one ($p = .058$), two ($p = .066$) or three ($p = .244$).

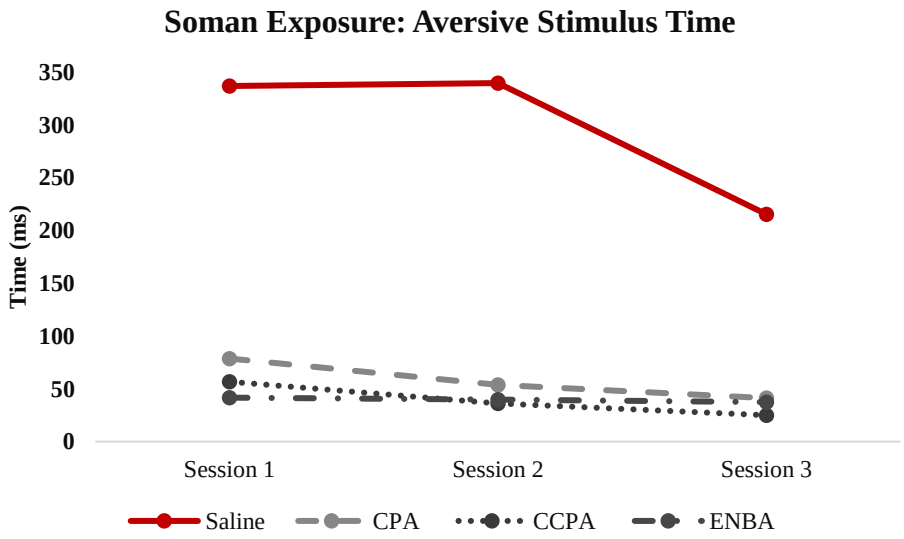

Figure 18.

Median Time of Exposure to Aversive Stimulus among Rats Challenged with Soman

Table 16.

Aversive Stimulus Time among Rats Challenged with Soman

Group	Treatment	*N*	Session	Median AS Time (ms)	Range
5	Saline	7	1	337.00	(264.74, 1007.65)
			2	339.68	(66.20, 747.04)
			3	215.35	(115.80, 1039.92)
6	CPA	10	1	78.70***	(47.75, 333.04)
			2	53.67*	(35.76, 891.88)
			3	41.20**	(0.00, 675.48)
7	CCPA	7	1	56.74**	(38.52, 368.12)
			2	36.17***	(0.00, 79.83)
			3	25.00***	(0.00, 45.13)
8	cdENBA	4	1	41.65**	(27.00, 60.80)
			2	39.89**	(32.44, 48.84)
			3	37.41**	(19.00, 79.96)

Statistically significant differences when comparing each A1AR agonist-treated group against the soman/saline control for each session are indicated by *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Neuropathology. Neuropathology was present in all regions of interest for every surviving animal from the soman/saline group ($N = 7$; *See Table 19 & Figure 21*). Of the ten surviving soman-exposed/CPA-treated rats, only one presented with neuropathology. Consequently, the animal was the only A1AR agonist-treated animal that developed SE then and survived the duration of the experiment to be eligible for histological evaluation. Neuropathology could not be observed in rats that did not survive to the experiment endpoint.

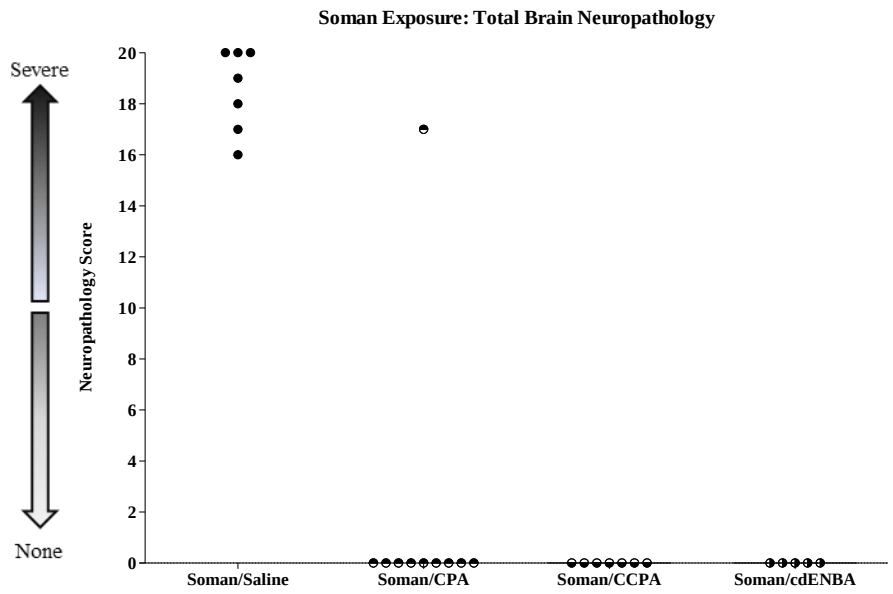

Figure 19.

Neuropathology Scores among Surviving Rats Challenged with Soman.

All surviving soman/saline animals demonstrated severe neuropathology in the regions of interest 14 days post-exposure. One surviving animal from the soman/CPA group developed SE during the initial experiment and, consequently, presented with severe neuropathology at the conclusion of the study. Surviving animals treated with A1AR-agonist compounds that effectively protected them from the onset of SE demonstrated no observable neuropathology.

Table 17.

Incidence of Neuropathology among Surviving Rats Challenged with Soman

Treatment	*N*	Pathology Present	Amygdala	Dorsal Hipp.	Ventral Hipp.	Piriform Cortex	Thalamus
Saline	7	7	100%	100%	100%	100%	100%
CPA	10	1	10%	10%	10%	10%	10%
CCPA	7	0	0%	0%	0%	0%	0%
cdENBA	4	0	0%	0%	0%	0%	0%

Discussion

The initial objective of this experiment was to establish a baseline for typical shuttle box performance (i.e., saline/saline control group) and examine the independent effect of A1AR agonism (following saline exposure) on that performance. Although animals in the saline/saline control group performed better than saline/A1AR agonist-treated groups during the first shuttle box testing session at 3 days post-administration, the trend did not continue in subsequent sessions at 7 and 14 days. This result, coupled with significant increases in the number of avoidance responses employed from the first to third session for all three A1AR agonist-treated groups, indicates that the proposed compounds had no enduring negative effect on the learning and memory processes required for avoidance learning as it was assessed in this study. Conversely, differences in shuttle box performance among saline/saline and soman/saline control groups (*See Figure 20*) validated the behavioral model by confirming observable alterations in the learning and memory processes of soman-exposed, saline-treated rats. These deficits in performance came to represent the baseline for typical behavior following soman intoxication that was used to evaluate the long-term value of the neuroprotection offered by CPA, CCPA and cdENBA.

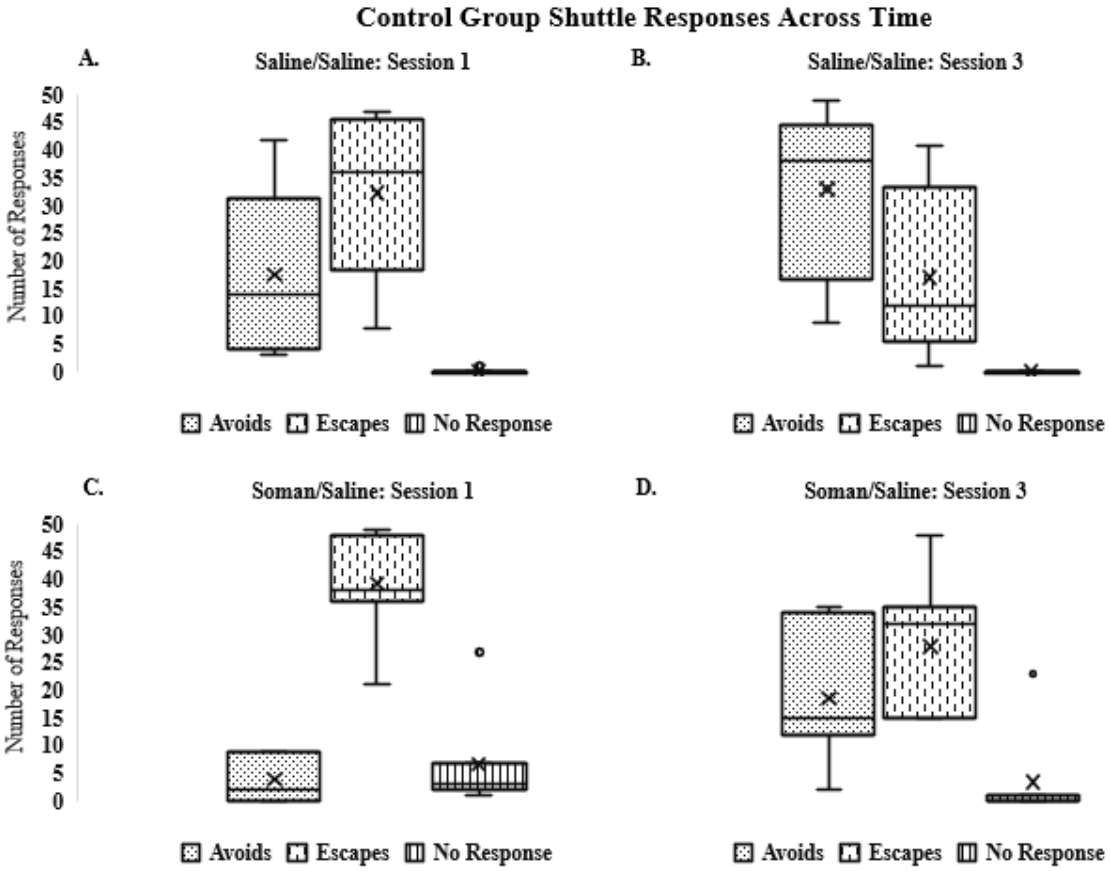

Figure 20.

Shuttle Responses among Saline/Saline and Soman/Saline Controls across Sessions.

Mann-Whitney *U* comparisons indicated significant differences in the number of avoidance responses employed by saline/saline (A) and soman/saline (C) groups during both session 1 ($p = < 0.01$) and session 3 (B & D; $p = < 0.01$).

Among soman-exposed animals, the saline-treated control group performed the poorest across all three behavioral sessions, suggesting that each A1AR agonist compound provided a level of protection of cognitive functioning not previously evaluated in ADO OP countermeasure studies. All soman-exposed, A1AR agonist-treated animals also regained normal righting ability faster than the soman/saline control group. Of the three agonists, CPA-treated animals took longest to regain their righting reflex but

illustrated that even the longest-acting A1AR agonist proposed in this study facilitated a decreased recovery time compared to the saline-treated control group. While CPA's longer inhibitory duration of action might seem like a negative pharmacological attribute, Thomas et al. (2018) proposed that it may allow for more prolonged suppression of damaging excitotoxic activity following OP exposure. Findings from this study support that theory as CPA was more efficacious in promoting 14-day survival than CCPA or cdENBA when administered after soman administration.

Interestingly, the only soman-exposed, A1AR agonist-treated animal that presented with neuropathology during the study was treated with CPA. However, it should be noted that the animal developed SE during the experiment and consequential neuropathology was to be expected. Presumably, all animals that were not adequately protected from SE (12/12 soman/saline, 3/12 cdENBA, 1/12 soman/CPA, and 2/12 CCPA; *See Figure 11)* would have demonstrated severe neuropathology had they survived the duration of the experiment.

CPA treatment was the only A1AR agonist treatment that resulted in an instance of 14-day survival despite the occurrence of SE. Not surprisingly, this unprotected animal demonstrated fewer avoidance responses and longer response times to the AS than the protected animals in the soman/CPA group, as well as a less consistent pattern of learning across time (*See Figures 21 & 22*). This isolated finding indicates that behavioral outcome as measured by a shuttle box assay is strongly related to effective seizure prevention. This discovery may also suggest that anticonvulsants (e.g., diazepam) prescribed by the current standard of care, which are incapable of consistently preventing

or terminating OP-induced SE, may also be unable to prevent (e.g., diazepam) long-term deficits in avoidance learning.

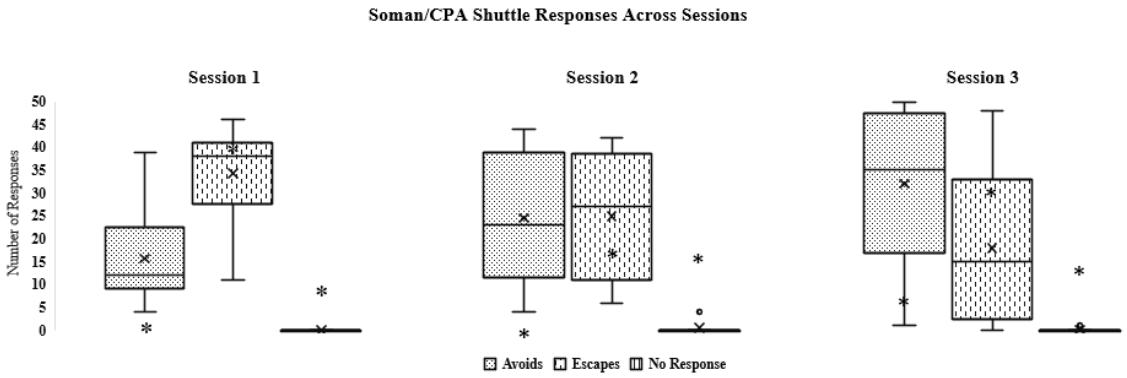

Figure 21.

Soman/CPA Shuttle Responses across Sessions.

Note. The numbers for avoids, escapes and non-responses demonstrated by the unprotected animal that developed *SE* and presented with severe neuropathology were removed from the group data (N = 9) represented. Instead, the numbers of avoids, escapes and non-responses for that animal (*N* = 1) are indicated with "*."

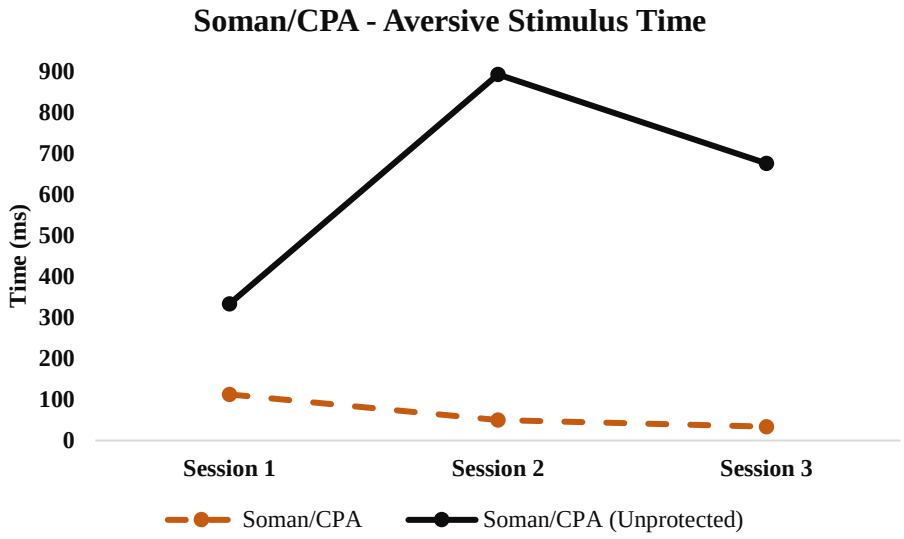

Figure 22.

Soman/CPA Median Shuttle Response to Aversive Stimulus across Sessions.

Note. The median aversive stimulus time for the unprotected animal that developed SE and presented with severe neuropathology has been removed from the group data ($N = 9$) represented (Soman/CPA). Instead, the median AS time experienced for the unprotected animal is represented individually (Soman/CPA (Unprotected); $N = 1$).

Physiological Outcomes and Limitations

All A1AR agonist compounds induced bradycardia and hypothermia during the experiment. Surviving animals did recover to a baseline range of heart rate and body temperature within 48 hours of soman exposure. Notably, while A1AR agonist-induced lethality was not observed in previous studies (Thomas et al., 2018), two saline-exposed/CPA-treated animals and one saline-exposed/CCPA-treated animal died before the study endpoint. The two CPA-treated animals appeared to be recovering with temperature readings of 88.56% and 98.67% of baseline, and heart rates that were 102.67% and 142.97% of baseline prior to death (2 & 3 days post-administration). However, their righting reflexes were still affected (scores of 1 & 2) at last recording (the

morning of experimental days 2 & 3, respectively). Unfortunately, even mildly sedated animals may be prone to asphyxiation and airway obstruction when not being monitored overnight. The CCPA-treated animal survived only the initial experimental day and displayed no righting ability (score of 3) before being returned to the home cage. The animal's temperature (79.51% of baseline) and heart rate (heart rate 24.68% of baseline) were among some of the lowest recorded for the group at the end of day one. Constant monitoring, external temperature regulation and nutritional support were not feasible within this animal model but will be explored moving forward.

Future Directions

Although the incidence of soman-induced SE was greatly reduced in response to CPA, CCPA and cdENBA treatment, some animals in each group were left unprotected. Previous work has shown that the route of administration utilized in this study (IP injection) may be vulnerable to human error, resulting in variable levels of A1AR agonist efficacy (Thomas et al., 2018). Future studies will evaluate intramuscular administration as an alternative to IP injection to minimize error and maximize systemic drug circulation. Considerations will also be made to regulate body temperature and provide nutritional support to better simulate a realistic model of care.

Moreover, while A1AR agonism following exposure to soman resulted in observable long-term benefits during this study, medical intervention for OP exposure includes the administration of centrally acting atropine sulfate, 2-PAM chloride and anticonvulsants (i.e., diazepam). To further evaluate the therapeutic potential for A1AR agonism as a countermeasure for OP exposure, the proposed compounds (i.e., CPA,

CCPA and cdENBA) should also be administered in conjunction with the treatments that compose the current standard of care.

Conclusion

Superior behavioral performance of A1AR agonist-treated animals following soman exposure indicates that peripherally delivered A1AR agonists administered one minute after exposure can prevent long-term deficits in associative learning and memory that are characteristic of OP intoxication. As expected, increased behavioral output translated to the absence of observed neuropathology across A1AR agonist-treated groups. Despite a more enduring effect on temperature and heart rate compared to CCPA or cdENBA-treatment, trends indicated that CPA was more efficacious in promoting 14-day survival, suggesting that off-target effects (i.e., reduction in heart rate and body temperature) associated with A1AR agonism may provide some therapeutic benefit. Furthermore, while A1AR agonist administration resulted in rapid, deep sedation, soman/A1AR agonist-treated animals regained their righting reflex faster and recovered weight faster than the soman/saline-treated control group, signaling enhanced physiological recovery. The findings of this study indicate that therapeutic augmentation of adenosine signaling is a promising alternative neuroprotective countermeasure.

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RESEARCH EXPERIENCE:

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Coordination and participation in neuroprotective, anticonvulsant animal model studies aimed at evaluating pharmacological interventions for nerve agent exposure.
Projects: (1) Determining minimum effective doses for adenosine agonist compounds delivered through different routes of administration (2) Optimizing seizure model and treatment regimens to promote survival (3)Verification of anti-seizure efficacy of acute and delayed adenosine agonism (4) Termination of sustained status epilepticus with adenosine agonist compounds (5) Examining long-term cognitive and behavioral deficits of nerve agent exposure and adenosine agonist treatments (6) Investigation of adenosine's anti-inflammatory potential (7) Evaluating candidate oxime compounds for central nervous system reactivation of acetylcholinesterase following nerve agent exposure
Skills: (1) Stereotaxic rodent surgeries, blood collection, injections, surgical microinjection, perfusions, brain extractions and dissections *(2)* Neurobehavioral and behavioral testing *(3)* Collection, input, and statistical analysis of experimental data *(4)* Conduction of ELISA and multiplex assays *(5)* Dilution and preparation of research compounds *(6)* Authorship and presentation of experimental findings

Fall 2018 — **Research Assistant**
Biopsychology Lab, Towson University | Towson, MD
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March 2019 — *Delayed adenosine A1 receptor agonist (±)-5'-Chloro-5'-deoxy-ENBA (cdENBA) treatment terminates soman-induced status epilepticus*
Meads, K., Wegener, A., Thomas, T., & Shih, T-S.

58th Annual Society of Toxicology Meeting & ToxExpo, Baltimore, MD

March 2019 *Intramuscularly Administered A1 Adenosine (ADO) Receptor Agonist (±)-5′-Chloro-5′-deoxy-ENBA (cdENBA) Induces Isoelectric Brain State in Rats*
Loughery, T., **Meads, K.**, Collazo-Martinez, Ana, Thomas, T., & Shih, T-S.
58th Annual Society of Toxicology Meeting & ToxExpo, Baltimore, MD

March 2019 *Crossing the blood-brain barrier to combat nerve agent*
Hornung, E., Acon-Chen, C., Berger, K., Coppola, J., Dunn, E., Hundertmark, K., Jackson Piercy, C., Lehman, J., Loughery, T., Martinez, A., **Meads, K.**, Mills, S., Moreno, J., Taylor, K., Wilson, S., McDonough, J., and Shih, T-S.
58th Annual Society of Toxicology Meeting & ToxExpo, Baltimore, MD

April 2019 *Acute adenosine A1 receptor agonist cdENBA treatment prevents neuropathology and cognitive deficits after soman exposure*
Meads, K., Wegener, A., Langston, J., Acon-Chen, C., Lehman, J., Myers, T., Thomas, T., & Shih, T-S.
21st Biennial Bioscience Review, Edgewood, MD (poster)

April 2019 *Developing a serum carboxylesterase knockout mouse model for in vivo reactivation of nerve agent-inhibited acetylcholinesterase*
Acon-Chen, C., Koenig, J., Lager, B., **Meads, K.**, Moreno, J., Treffalls, J., McMonagle, J., Ballough, D., Dunn, E., Ardinger, C., Haines, K., Jackson, C., Cadieux, C., Cerasoli, D., McDonough, J., & Shih, T-S.
21st Biennial Bioscience Review, Edgewood, MD (poster)

April 2019 *Resilience as a predictor of physiological response to stress*
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TEACHING EXPERIENCE:

Spring 2019 **Graduate Teaching Practicum**
Psychophysiology | Towson University, Towson, MD
Mentor: Dr. Mark Chachich
Planned and taught several lessons, lead exam review sessions, graded exams and weekly assignments, answered student questions, held weekly office hours

Fall 2016 **Teaching Assistant**
Personality Theory | Towson University, Towson, MD
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Administered exams, graded exams and weekly assignments, provided student tutoring, held weekly office hours

PROFESSIONAL EXPERIENCE:

2014 – 2017	Department Head, Client Relations Team *Mid Atlantic Benefits Group	Havre de Grace, MD*
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- AcqKowledge 4.4
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