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Characterizing the behavioral effects of nerve agent-induced seizure activity in rats: Increased startle reactivity and perseverative behavior☆,☆☆

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ABSTRACT

The development and deployment of next-generation therapeutics to protect military and civilian personnel against chemical warfare nerve agent threats require the establishment and validation of animal models. The purpose of the present investigation was to characterize the behavioral consequences of soman (GD)-induced seizure activity using a series of behavioral assessments. Male Sprague–Dawley rats (n = 24), implanted with a transmitter for telemetric recording of encephalographic signals, were administered either saline or 1.0 LD_{50} GD (110 μg/kg, sc) followed by treatment with a combination of atropine sulfate (2 mg/kg, im) and the oxime HI-6 (93.6 mg/kg, im) at 1 min post-exposure. Seizure activity was allowed to continue for 30 min before administration of the anticonvulsant diazepam (10 mg/kg, sc). The animals that received GD and experienced seizure activity had elevated startle responses to both 100- and 120-dB startle stimuli compared to control animals. The GD-exposed animals that had seizure activity also exhibited diminished prepulse inhibition in response to 120-dB startle stimuli, indicating altered sensorimotor gating. The animals were subsequently evaluated for the acquisition of lever pressing using an autoshaping procedure. Animals that experienced seizure activity engaged in more goal-directed (i.e., head entries into the food trough) behavior than did control animals. There were, however, no differences between groups in the number of lever presses made during 15 sessions of autoshaping. Finally, the animals were evaluated for the development of fixed-ratio (FR) schedule performance. Animals that experienced GD-induced seizure activity engaged in perseverative food trough-directed behaviors. There were few differences between groups on other measures of FR schedule-controlled behavior. It is concluded that the GD-induced seizure activity increased startle reactivity and engendered perseverative responding and that these measures are useful for assessing the long-term effects of GD exposure in rats.

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1. Introduction

Soman (GD; pinacolyl methyl phosphonofluoridate) is a highly toxic organophosphorus (OP) compound that was originally developed as a chemical warfare nerve agent and still represents a major threat to both military and civilian personnel. The toxic effects of GD are primarily due to the irreversible inhibition of the enzyme acetylcholinesterase (AChE), resulting in the accumulation of acetylcholine (ACh) at the synapse and neuromuscular junction and over-stimulation of the cholinergic system. GD inhibition of AChE occurs through the binding of GD at the active serine site of AChE. Once bound, this complex rapidly undergoes dealkylation ("aging"), resulting in a stable monoalkyl-phosphonylated complex with AChE, and resumption of normal AChE activity requires de novo synthesis (reviewed in Marrs et al., 2006). The central nervous system (CNS) effects of nerve agents in humans include giddiness, anxiety, restlessness, headache, tremor, confusion, failure to concentrate, convulsions, respiratory depression, and respiratory arrest (Marrs, 2007).

The rapid inhibition of AChE and subsequent increase in synaptic ACh levels can lead to the development of seizure activity that can rapidly progress to *status epilepticus* (de Araujo Furtado et al., 2010; McDonough and Shih, 1997; McDonough et al., 2009). If the seizure activity is left untreated, profound brain damage can occur (Baille et al., 2005; McDonough and Shih, 1997; Shih et al., 2003). In fact, McDonough et al. (1995) showed that at least 20 min of seizure activity is necessary for neuropathological damage to occur in rats following nerve agent exposure. Nerve agent-induced seizures produce the most pronounced neuropathology in the piriform cortex, thalamus, amygdala,

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☆☆ The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals* and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

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and hippocampus (Apland et al., 2010; Baille et al., 2001, 2005; Collombet et al., 2005; Filliat et al., 1999; Kadar et al., 1995; Lemercier et al., 1983; McDonough et al., 1986, 1998; McLeod, 1985; Modrow and Jaax, 1989; Petras, 1981, 1994; Raveh et al., 2002, 2003; Shih et al., 2003; Tryphonas and Clement, 1995) and contribute to long-term behavioral and cognitive deficits (Brandeis et al., 1993; Buccafusco et al., 1990; Collombet et al., 2008; Coubard et al., 2008; Filliat et al., 2007; Raffaele et al., 1987; Raveh et al., 2002, 2003).

There are numerous reports of behavioral deficits resulting from seizure-inducing levels of GD exposure. McDonough et al. (1986) reported a significant negative correlation between the severity of GD-induced neuropathology and the rate of acquisition of DRL (differential reinforcement of low rate responding) schedule performance. Haggerty et al. (1986) examined the acoustic startle response (ASR) of rats in response to GD challenge and reported decreased startle magnitude at 2 h following exposure to 0.8 LD_{50} (150 μg/kg, im) GD; however, they did not assess the startle response at later time points. In contrast, Philippens et al. (2000, 2005) reported elevated ASRs in guinea pigs at 2 and 24 h following 2.0 LD_{50} (49 μg/kg, sc) GD exposure. Joosen et al. (2009) reported mnemonic impairments in the Morris water maze at 8 weeks following 1.8 LD_{50} (200 μg/kg, sc) GD exposure in rats. Coubard et al. (2008) observed anxiety-like behaviors in mice at 30 and 90 days following 1.2 LD_{50} (110 μg/kg, sc) GD exposure. Auditory and contextual fear conditioned responses were also increased in these mice at 30 days post-exposure. On the other hand, Moffett et al. (2011) observed a severe impairment in auditory and contextual fear conditioning at approximately 1 week following 1.0–1.2 LD_{50} (110–132 μg/kg, sc) GD exposure in rats. Differences in species, time span, and neuropathology may account for the discrepancies between some of these reports of GD-induced behavioral deficits.

The purpose of the present study was to investigate the effects of GD-induced seizure activity on a series of behavioral tests (see Table 1). Three different behavioral procedures were chosen for inclusion in this experiment. First, ASR and reflex modification techniques (prepulse inhibition, PPI) (Davis, 1984) were chosen because these procedures have been used in both the rat (Haggerty et al., 1986) and guinea pig (Philippens et al., 2000, 2005) models of GD exposure, and lesions of the basolateral amygdala (Wan and Swerdlow, 1997) and the entorhinal cortex (Goto et al., 2002) have been shown to reduce PPI in rats without changing startle amplitude. However, these unconditioned behaviors have not been systematically evaluated in animals exposed to seizure-inducing levels of GD. Second, we chose to investigate the acquisition of lever-pressing using an autoshaping procedure (Sparber, 2001). Lesion studies have demonstrated that limbic structures typically damaged by GD-induced seizures (i.e., hippocampus) are necessary for the development of autoshaped responding in multiple species (Good and Honey, 1991; Hall et al., 1996; Reilly and Good, 1989; Richmond and Colombo, 2002). Furthermore, these procedures have been used extensively to detect the effects of neurotoxic compounds (Cohen et al., 1987; Fossom et al., 1985; Messing et al., 1988). Finally, we chose to evaluate the development of fixed-ratio (FR) schedule performance and the animals' abilities to adapt to changing reinforcement requirements. These techniques have been shown to be sensitive to the effects of a wide range of neurotoxic chemicals (Cory-Slechta, 1986; Gentry and Middaugh, 1988; Gerbec et al., 1988; Hojo et al., 2002; Middaugh and Gentry, 1992; Newland et al., 1986, 1994; Paletz et al., 2006), and Rabe and Haddad (1968) showed that hippocampal lesions in rats increased responding under an FR 20 schedule. The results of these experiments will be used to characterize nerve agent-induced seizure-related behavioral deficits.

Table 1
Sequence of phases, conditions, number of sessions, and the post-exposure day of testing.

Phase	Condition	Schedule	Number of sessions	Post-exposure day[a]
ASR	Baseline (pre-exposure)		3	
	Post-exposure		3	7–9
Food restriction				15–16
Operant acquisition	Magazine training		2	17–18
	Autoshaping		15	22–42
	Lever press training		4	43–46
	Reinforcement equalization		5	47–53
	Fixed ratio	FR 1	3	56–58
		FR 5	3	59–64
		FR 25	3	65–67
		FR 75	3	70–72
		FR 5	3	73–77

[a] Post-exposure days were counted from the first day of exposure.

2. Methods

2.1. Subjects

Twenty-four adult male Sprague–Dawley rats (pre-exposure weights: mean 475 g, range 422–563 g) were obtained from Charles River Laboratories (Kingston, NY, USA). Upon arrival, they were acclimated for 5 days and observed for evidence of good health. Animals were housed individually in polycarbonate cages in a temperature (21 ± 2 °C) and humidity (50 ± 10%) controlled colony room maintained on a reversed 12-h light–dark cycle with lights off at 0900 h. All experimental manipulations were conducted during the dark phase of the light–dark cycle when the animals are the most active. Food and water were available ad libitum in home cages. Animals were allowed to acclimate to the colony room (>1 week) before experimental procedures began. One week prior to the autoshaping phase (see Table 1 and below), the animals were placed under caloric regulation. This consisted of allotting the animals an amount of food equal to 90% of their estimated daily energy requirements (112 kcal/body weight $^{0.75}$) (Subcommittee on Laboratory Animal Nutrition, 1995). When applicable, the animals were fed at least 1 h following testing sessions. Water was available ad libitum in the home cage.

2.2. Surgery

2.2.1. Transmitter implantation

Approximately 1 week before experimentation, 16 animals were implanted with transmitters (F40-EET; Data Science International, St. Paul, MN, USA) to record electroencephalographic (EEG) activity and body temperature. The animals were anesthetized with isoflurane (3% induction; 1.5–2% maintenance with oxygen) and placed in a stereotaxic apparatus. One pair of cortical screws was placed bilaterally 2 mm from midline and 4 mm caudal to bregma. A second pair was placed 2 mm from midline and 1.5 mm rostral relative to lambda. The transmitters were implanted midscapular (sc), and the electrodes passed sc and wrapped around the cortical screws before being encased in dental acrylic. The incisions were sutured and treated with topical antibiotic ointment. For additional methods on transmitter implantation, see Williams et al. (2006). Animals were removed from the stereotaxic apparatus, placed on a circulating hot water blanket until consciousness was regained, and given buprenorphine (0.05 mg/kg, sc) before being returned to the colony room. Since there were a limited number of transmitters, the remaining 8 animals underwent sham surgeries. The sham surgeries were identical to the transmitter implantation surgeries with the exception that no transmitter was implanted. All animals were allowed 1 week to recover before further experimental manipulations were performed.