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Repeated low-dose exposures to sarin, soman, or VX affect acoustic startle in guinea pigs

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ABSTRACT

Chemical warfare nerve agents (CWNAs) are known to cause behavioral abnormalities in cases of human exposures and in animal models. The behavioral consequences of single exposures to CWNAs that cause observable toxic signs are particularly well characterized in animals; however, less is known regarding repeated smaller exposures that may or may not cause observable toxic signs. In the current study, guinea pigs were exposed to fractions (0.1, 0.2, or 0.4) of a medial lethal dose (LD_{50}) of sarin, soman, or VX for two weeks. On each exposure day, and for a post-exposure period, acoustic startle response (ASR) was measured in each animal. Although relatively few studies use guinea pigs to measure behavior, this species is ideal for CWNA-related experiments because their levels of carboxylesterases closely mimic those of humans, unlike rats or mice. Results showed that the 0.4 LD_{50} doses of soman and VX transiently increased peak startle amplitude by the second week of injections, with amplitude returning to baseline by the second week post-exposure. Sarin also increased peak startle amplitude independent of week. Latencies to peak startle and PPI were affected by agent exposure but not consistently among the three agents. Most of the changes in startle responses returned to baseline following the cessation of exposures. These data suggest that doses of CWNAs not known to produce observable toxic signs in guinea pigs can affect behavior in the ASR paradigm. Further, these deficits are transient and usually return to baseline shortly after the end of a two-week exposure period.

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1. Introduction

Exposure to organophosphorus (OP) cholinesterase inhibitors including the chemical warfare nerve agents (CWNA) sarin (GB), soman (GD), and VX induces central and peripheral nervous system toxicity primarily by irreversibly binding acetylcholinesterase (AChE). Documented cases of attacks with CWNAs include those in the Iran-Iraq war (Balali-Mood and Saber, 2012), the 1994 and 1995 Japan subway attacks (Miyaki et al., 2005), and the Gulf War (Proctor et al., 2006). The recent CWNA attacks in Syria highlight the continued need for a better understanding of, and countermeasures for, these agents (Rosman et al., 2014). In addition to CWNAs, the widespread use of OP pesticides, particularly in the developing world, accounts for millions of poisonings each year (Karalliedde and Senanayake, 1989). Acute symptoms of OP intoxication include miosis, salivation, gastrointestinal disturbances, tremors, seizures, and respiratory failure (Newmark, 2007). Moderate-to-high doses produce well characterized physiological and behavioral effects; however, repeated exposures to relatively low levels of OP pesticides or CWNAs can result in muted or transient symptoms, the consequences of which are poorly understood.

Instances of past low-level exposures in humans underscore the poor understanding of these agents' behavioral effects, particularly in individuals that do not exhibit observable toxic signs at the time of exposure. In the 1995 Tokyo sarin attack, reports describe neurobehavioral deficits in individuals exposed to non-lethal doses of sarin (Nishiwaki et al., 2001; Yokoyama et al., 1998), but subjects in these cases were symptomatic (Okumura et al., 2005). In another example, the Gulf War conflict in the 1990s, warfighters suspected of being exposed to sarin and cyclosarin exhibited neurobehavioral symptoms 4–5 years after returning from deployment (Proctor et al., 2006), even though no observable signs of CWNA intoxication were known to be recorded during the conflict (Riddle et al., 2003). Although the Gulf War example suggests an association between repeated low-level CWNA exposure and behavioral dysfunction, determining the extent to which CWNAs contributed is difficult because of the presence of pollutants, cholinesterase medications, and psychological stress in theater (Riddle et al., 2003; White et al., 2015).

Animal models provide one method of assessing the unique consequences of repeated low-dose CWNA exposures on behavior. In Atchison et al. (Atchison et al., 2004), the effects of repeated daily exposures (Monday–Friday) to fractions (0.1, 0.2, 0.4, 0.6, or 0.8) of the

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established median lethal dose (LD_{50}) of GD, GB, or VX (28, 42, or 9 μg/kg, sc, respectively) over 4 weeks were measured to determine the maximum tolerated dose (MTD) of each agent in guinea pigs as measured by acute symptoms of exposure, decrements in body temperature, changes in body weight, or gross pathology. Exposure to 0.6 or 0.8 LD_{50} of these agents produced toxic signs within minutes, whereas lower doses did not, suggesting that 0.4 LD_{50} was the MTD for repeated daily exposures up to 4 weeks (Atchison et al., 2004). Similar effects extend to mice and rats; daily injections of 0.4 LD_{50} of GB (64 μg/kg, sc) for three days in mice (Mach et al., 2008) and repeated exposures of inhaled GB in rats (1.25 μg/L, three exposures per week; Kassa et al., 2001) produced no observable toxic signs. Although physiological effects of a repeated MTD have been characterized for a variety of CWNAs in several species, further systematic investigation is required to understand the behavioral effects resulting from repeated exposure to low levels of CWNAs.

Historically, behavioral disruptions following CWNA intoxication have depended largely on the animal species, agent, dose, and latency to perform behavioral tests. Previous studies have indicated that a single exposure to GB and GD well above the MTD could disrupt behavior in most animal models, particularly when the dose was high enough to produce severe toxic signs to include seizures (Gilat et al., 2005; Langston et al., 2012). In guinea pigs, with 1 LD_{50} of GD being greater than 24.5 μg/kg (sc) and behavioral testing occurring at 24 h after exposure, a single 2 LD_{50} dose of GD increased peak startle amplitude in the acoustic startle paradigm and decreased the number of correct avoidance responses in the shuttlebox test (Philippens et al., 2000). Additionally when testing occurred 3 months post-exposure, 1 LD_{50} of GD increased the latency to locate an escape platform in the Morris water maze (Mamczarz et al., 2011). In rats, from one to eleven weeks post-exposure (depending on the behavioral test), a single exposure to 1 LD_{50} of GD (110 μg/kg, sc) decreased the duration of freezing in contextual and auditory conditioning tests (Moffett et al., 2011), increased peak startle amplitude in the acoustic startle paradigm, diminished pre-pulse inhibition (PPI), and potentiated perseverative behaviors in operant tasks (Langston et al., 2012). Similarly, a single exposure to 1.2 LD_{50} of GB (108 μg/kg, im) in rats disrupted memory acquisition in the Morris water maze at one week post-exposure (Gilat et al., 2005; Raveh et al., 2008). Such deficits were associated with neuronal damage to brain regions, including the thalamus, hippocampus, amygdala, and piriform cortex (Langston et al., 2012; Moffett et al., 2011).

Although the effects of high doses are well established, behavioral disruptions following single doses below 1 LD_{50} are equivocal. In rats, whole-body exposures to GD, GB, or VX for 60 min of approximately 0.53 of the median lethal concentration (LC_{50}) (7, 4, and 0.45 mg/m^3, respectively) 48 h before testing had transiently small or no effects on response rates to food-reinforced operant tasks or memory acquisition in the radial arm maze (Genovese et al., 2009a; Genovese et al., 2007; Genovese et al., 2009b). When testing occurred 45 min after exposure, injections of ~0.1 LD_{50} (50 μg/kg, ip) of GB in rats disrupted locomotion and rearing in an open field (Nieminen et al., 1990), while 0.59 LD_{50} (98 μg/kg, sc) of GB affected rotorod performance (Landauer and Romano, 1984). A dose of 0.3 LD_{50} of GB (37 μg/kg, sc), however, did not disrupt active avoidance in the shuttlebox paradigm or exploration in the open field (Wolthuis and Vanwersch, 1984). A 0.3 LD_{50} of GD (46 μg/kg, sc) affected some tests but not others, although the authors note that these differences were confounded by changes in dietary composition (Wolthuis and Vanwersch, 1984). Unlike the behaviors described in (Wolthuis and Vanwersch, 1984), doses of GD below 0.3 LD_{50} in mice revealed differences in the elevated plus maze, such that 0.27 LD_{50} of GD (30 μg/kg, sc) *decreased* anxiety-related behaviors in the elevated plus maze 30 min but not 24 h or 7 days post-exposure. Interestingly, 0.45 LD_{50} of GD (50 μg/kg, sc) in mice *increased* anxiety-related behaviors at the same 30-min time point, which highlights the potentially complicating effect of dosing CWNAs when measuring behavior (Baille et al., 2001).

The effects of repeated exposures close to the MTD (0.5 LD_{50} or less) of CWNAs are also inconsistent and likely depend on similar factors observed after single exposures. Daily exposures to 0.53 LC_{50} of GB in rats for three days depressed the response rate for a food reinforcement, which returned to control rates later on the same day (Genovese et al., 2009b). Continuous administration of 0.05 LD_{50} (2.25 μg/kg, sc) per

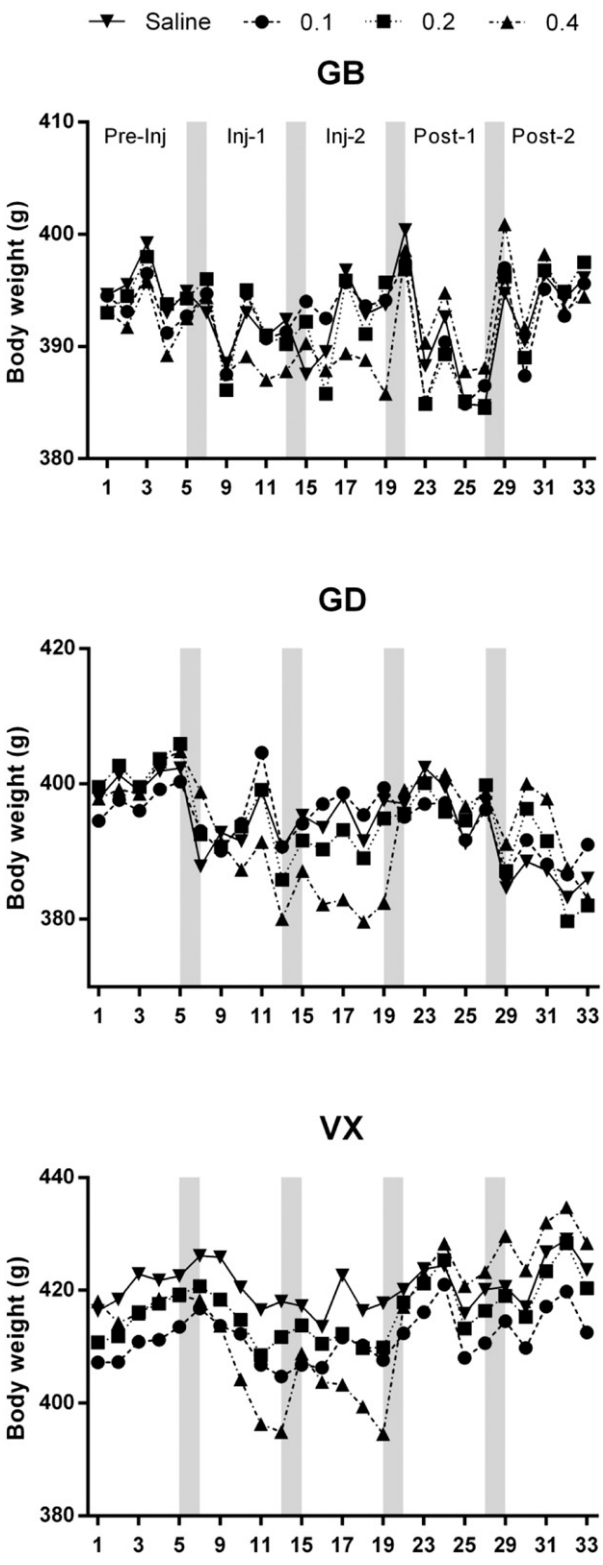

Fig. 1. Body weights for guinea pigs exposed to GB (*top*), GD (*middle*), and VX (*bottom*). Gray bars represent weekends when body weight data were not recorded.