



Behavioral side effects of prophylactic therapies against soman-induced seizures and lethality in rats



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ABSTRACT

Four medical therapies previously shown to exert varying degrees of protection against a convulsant dose of soman were assessed for potential behavioral side effects in a novelty test. In Experiment 1, HI-6 (1-[[4-(aminocarbonyl)pyridino] methoxy)methyl]-2-[(hydroxyimino)methyl]pyridinium) (125 mg/kg), scopolamine (1 mg/kg), physostigmine (0.1 mg/kg), levetiracetam (50 mg/kg), and procyclidine (20 mg/kg) were tested separately. In Experiment 2, the combination of HI-6, scopolamine, and physostigmine (termed the physostigmine regimen) or HI-6, levetiracetam, and procyclidine (termed the procyclidine regimen) were tested. In Experiment 3, the metabotropic glutamate modulators DCG-IV ((2S,2'R,3'R)-2-(2',3'-dicarboxycyclopropyl)glycine) (4 mg/kg) and MPEP (2-Methyl-6-(phenylethynyl)pyridine hydrochloride) (30 mg/kg) were tested separately or each drug in combination with HI-6 and procyclidine (termed the DCG-IV regimen and the MPEP regimen, respectively). The results showed that the physostigmine and procyclidine regimens both produced severe cognitive impairment (lack of preference for novelty) and reduced locomotor and rearing activities. The DCG-IV and MPEP regimens caused milder deficits on the same behavioral measures. Some relations were seen between prophylactic capacity and degree of behavioral side effects. Only HI-6 or levetiracetam had no adverse effects on behavior. DCG-IV or MPEP produced some impairment, whereas the detrimental effects of scopolamine or procyclidine were pronounced. The relatively high dose of procyclidine (anticholinergic and antiglutamatergic) needed for prophylactic efficacy may have played a major role for the side effects of the regimens in which the drug was used. It was concluded that behavioral side effects are inevitable for potent prophylactic therapies against soman intoxication.

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1. Introduction

Exposure to nerve agent requires fast treatment with antidotes, because such organophosphorus toxicant causes dramatic enhancement of cholinergic activity in all

bodily organs, most notably the central nervous system. In the brain, the cholinergic overstimulation rapidly triggers excitotoxic glutamatergic activity accompanied by seizures subsequently leading to death, because the regulatory action of the respiratory center in the brainstem is interrupted by the high level of electrical discharges [1]. The excessive cholinergic activity initiated by nerve agent causes irreversible inhibition of acetylcholinesterase, the enzyme that hydrolyzes acetylcholine.

In order to prevent lethality by nerve agent it is important to shield temporarily a portion of the

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acetylcholinesterase from irreversible inhibition. Following exposure, therapeutic treatment with an anticholinergic drug is supposed to be given. To meet these requirements, a number of military forces have based their medical therapy on pyridostigmine pretreatment to prevent acetylcholine inhibition by nerve agents followed by the immediate therapeutic treatment with atropine sulfate and an oxime administered by one or more autoinjectors. These drugs are intended to inhibit muscarinic receptors and to reactivate any “unaged” enzyme, respectively, following exposure to nerve agent [2]. However, inasmuch as pyridostigmine does not readily cross the blood-brain barrier, physostigmine that readily enters the brain, has been suggested as a possible replacement. In studies of guinea pigs and rats, evidence has been presented that effective prevention of soman-induced lethality can be ensured by physostigmine in combination with scopolamine or procyclidine [3–7]. Pyridostigmine combined with caramiphen or benactyzine and trihexyphenidyl or with biperiden have also been reported to provide efficacious pretreatment in soman-poisoned rats [8–10].

Two medical therapies consisting of HI-6 (1-[[4-(aminocarbonyl)pyridino] methoxy)methyl]-2-[(hydroxyimino)methyl]pyridinium), scopolamine, and physostigmine (termed the physostigmine regimen) or HI-6, levetiracetam, and procyclidine (termed the procyclidine regimen) have been shown to be very effective 1 min after high levels of soman poisoning, but they differ markedly in anticonvulsant capacity when administered 10 or 20 min after soman exposure. However, when given 20 min before soman intoxication, both regimens exert very effective protection [11]. Furthermore, in a study of the metabotropic glutamate modulators DCG-IV ((2*S*,2'*R*,3'*R*)-2-(2',3'-dicarboxycyclopropyl)glycine) and MPEP (2-Methyl-6-(phenylethynyl)pyridine hydrochloride) it was demonstrated that treatment regimens consisting of HI-6, procyclidine, and DCG-IV (termed the DCG-IV regimen) or HI-6, procyclidine, and MPEP (termed the MPEP regimen) both have substantial antidotal efficacy when administered 20 min after onset of soman-induced seizures. When given 20 min before challenge with soman, the DCG-IV regimen provides good protection, whereas the MPEP regimen has weaker antidotal impact [12]. In the present study, it was of interest to examine whether the degree of protective potency of the various regimens may be related to the degree of potential behavioral side effects produced by the regimens.

Because seizures are associated with both lethality and brain damage [13], it is very important to prevent the onset of seizures or terminate seizures within 20 min after onset to avoid neuropathology [14,15]. However, a crucial matter is whether the doses of prophylactics required for protection of military personnel against nerve agent-induced damage will impair cognitive functions. The purpose of the present study was to make a comparative assessment of potential behavioral effects of HI-6, scopolamine, physostigmine, levetiracetam, and procyclidine separately (Experiment 1), the combinations of HI-6, scopolamine, and physostigmine or HI-6, levetiracetam, and procyclidine (Experiment 2), DCG-IV and MPEP separately or each drug in combination with HI-6 and procyclidine

(Experiment 3). The doses of drugs chosen have previously been shown to have anticonvulsant effects against soman-induced seizures. The behavioral task employed was a novelty test that has proven particularly sensitive in revealing cognitive dysfunctions following selective disruptions of entorhinal projections [16,17]. Exploration of a discrete novel object is one form of inquisitive activity frequently seen among rats. This activity appears as a strong preference for novelty, the recognition of which is probably based on polymodal sensory information [18]. The rats were tested in a modified version of the novelty test of Berlyne [19] consisting of three different sets of stimuli: visual/tactile, olfactory, or visual only [16].

2. Materials and methods

2.1. Animals

2.1.1. Experiment 1

Forty-eight male Wistar albino rats from a commercial supplier (Taconic Breeding Laboratories, Denmark) weighing 280–310 g when the experiment started, served as subjects. The rats were randomly assigned to one of the 6 groups (8 rats in each) and their group assignment was unknown during testing. The various groups received either saline, HI-6, scopolamine, physostigmine, levetiracetam, or procyclidine. The rats were housed individually and had free access to commercial rat pellets and water. With the novelty test used, reliable results are dependent on emotionally stable animals. For this reason, the rats were handled individually 7–10 days, being allowed to explore a table top (80 cm × 60 cm) for 3 min a day. The climatized (21 °C) vivarium was illuminated from 0700 to 1900 h.

2.1.2. Experiment 2

Twenty-four male Wistar rats (280–310 g) from the same supplier served as subjects. The animals were randomly assigned to one of the 3 groups with 8 rats in each. The various groups received saline, the combination of HI-6, scopolamine, and physostigmine or the combination of HI-6, levetiracetam, and procyclidine. The rats were treated as described for Experiment 1.

2.1.3. Experiment 3

Forty male Wistar rats (280–310 g) from the same supplier served as subjects. The animals were randomly assigned to 5 groups with 8 rats in each. The various groups received saline, DCG-IV, MPEP, or the combinations of HI-6, procyclidine, and DCG-IV or HI-6, procyclidine, and MPEP. The rats were treated as described for Experiment 1.

The experiments were approved by the National Animal Research Authority. A minimal number of animals were used, and all efforts were made to avoid animal suffering according to The Code of Ethics of the World Medical Association and the EU Directive 2010/63/EU.

2.2. Drug administration

The drug doses chosen for Experiment 1 and 2 were derived from previous studies of anticonvulsant effects against soman-evoked seizures in rats: HI-6

Table 1

Mean (\pm SEM) measures of exploratory behavior in seconds in novelty test in Experiment 1. Differential time exploring is the difference between exploring novel and neutral items.

Group	N	Dose mg/kg	Differential time exploring			Total time exploring					
			Session			Session					
			I	II	III	I	II		III		
			Phase 2	Phase 2	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2
Saline	8	–	8.5 \pm 2.1	21.2 \pm 4.0	15.4 \pm 2.2	13.8 \pm 1.9	14.1 \pm 5.7	16.4 \pm 1.7	34.1 \pm 8.3	13.4 \pm 2.8	21.3 \pm 3.9
HI-6	8	125	8.3 \pm 2.2	19.1 \pm 3.6	13.9 \pm 3.0	23.8 \pm 3.0	23.1 \pm 3.6	17.8 \pm 2.3	35.3 \pm 3.6	28.5 \pm 4.5 ^b	25.8 \pm 4.1
Scopolamine	8	1	0.5 \pm 0.3 ^a	2.6 \pm 0.5 ^b	1.2 \pm 0.8 ^c	12.4 \pm 2.3	10.0 \pm 2.5	3.1 \pm 1.1 ^b	4.3 \pm 2.1 ^c	1.5 \pm 0.4 ^a	6.1 \pm 2.0 ^b
Physostigmine	8	0.1	4.8 \pm 1.5	17.5 \pm 3.6	16.3 \pm 3.1	17.8 \pm 2.3	9.4 \pm 1.9	16.4 \pm 3.5	20.3 \pm 3.3	19.1 \pm 4.5	23.0 \pm 3.6
Levetiracetam	8	50	7.8 \pm 2.4	23.9 \pm 5.4	10.6 \pm 2.2	16.4 \pm 3.8	15.8 \pm 4.3	12.1 \pm 3.3	26.9 \pm 5.4	14.5 \pm 2.1	17.0 \pm 2.4
Procyclidine	8	20	1.2 \pm 0.4 ^a	3.7 \pm 1.4 ^b	2.8 \pm 0.5 ^b	15.0 \pm 2.6	8.3 \pm 2.6	12.0 \pm 3.4	8.9 \pm 1.9 ^b	3.1 \pm 1.7 ^a	4.7 \pm 0.8 ^b

Significantly different from the saline group:

^a $p < 0.05$,

^b $p < 0.01$,

^c $p < 0.001$.

dimethanesulfonate 125 mg/kg, scopolamine hydrobromide 1 mg/kg, physostigmine salicylate 0.1 mg/kg, levetiracetam (Keppra®) 50 mg/kg, procyclidine hydrochloride 20 mg/kg [11,20–22]. The drugs chosen for Experiment 3 were based on a previous study of anticonvulsant effects: DCG-IV 4 mg/kg and MPEP 30 mg/kg [12]. The doses of HI-6 and procyclidine were the same as for Experiment 1 and 2. The drugs were dissolved in 0.9% saline. MPEP was dissolved in isotonic saline at a concentration of 5 mg/ml. All drugs were administered intraperitoneally (i.p.). The drugs were given 20 min before each test session (one session a day for 3 days) with a total testing period of 20 min. When combinations were made, the injections were given in rapid succession according to the sequences of drugs presented in Tables 3 and 5. Physiological saline was injected i.p. in a volume of 0.3 ml. Prophylactics are usually given 20 or 30 min before exposure to nerve agent [23]. Scopolamine, physostigmine, and procyclidine were purchased from Sigma (St Louis, MO, USA). DCG-IV and MPEP were purchased from Tocris Cookson Ltd (Bristol, UK), and HI-6 DMS was a generous gift from Defence Research and Development (Suffield, Medicine Hat, Canada). Levetiracetam is commercially available as Keppra®.

2.3. Apparatus

Behavioral testing was carried out in a Plexiglas cage (54 cm \times 33 cm \times 20 cm) previously described [16]. In brief, the floor was divided in 18 equal squares (9 cm \times 11 cm). Three identical aluminum cubes (5 cm \times 5 cm \times 5 cm) were evenly distributed in the cage in fixed positions (the neutral objects). Three other cubes made up the novel objects. One object only differed from the neutral ones in that its top was uneven with tracks (2 mm) in it making up a square pattern (visual/tactile stimuli). Since the rats could perceive the tracks or the squares (16 squares measuring 1.1 cm \times 1.1 cm) by bodily contact, both tactile and visual sensory modalities might be used. One was identical with the neutral ones, and a spot of cheese (dia. 1.5 cm) was smeared on the side facing the experimenter (olfactory stimulus). So-called Norwegian white cheese (Norvegia)

that hardly smells at all to humans was used. In the test cage, it was not possible to detect the cheese visually. One was smaller than the neutrals (4.5 cm \times 4.5 cm \times 4.5 cm) and two sides were slightly uneven (visual stimulus). All objects were painted light gray. The sound attenuated testing room was provided with a fan producing white noise (52 dB).

2.4. Procedure

The same procedure was followed for Experiments 1, 2 and 3. During adaptation, the rats were allowed to explore individually the empty apparatus for 20 min. On the next day, the rats were given the test drugs before they were run in Session I. In Phase 1, the animals were tested for 5 min in the test cage with three neutral objects present. Then the rats spent 10 min in the home cage. In Phase 2, the rats were tested again for 5 min, and the neutral object in the middle position had been replaced by the novel object with uneven top. Changing position of neutral object makes up a novelty in itself [24]. Preference for novelty was based on the difference between exploration of novel versus neutral objects, and the mean time of contact with the two neutral objects was used. During Phases 1 and 2 the following behaviors were recorded: number of seconds in contact with the objects, number of squares traversed (locomotor activity), and number of rearings. Exploration of an object was defined as directing the snout toward the object at a distance of 1.5 cm or less. Bodily touch other than by the snout was not considered as exploratory behavior. Prior to testing of each rat the apparatus and objects were carefully washed with Zalo (Lilleborg, Norway) dissolved in water and allowed to dry. In Sessions II and III (test days 2 and 3), the same procedure was followed, and the novelty was represented by smell of cheese on one side of the cube and a smaller object, respectively. Since changing the order of novelty presentation can lead to different patterns of locomotor and rearing activity, a counterbalanced order of testing was not used to control for accumulative effects of drugs on activity measures. The same set of neutral cubes was used after olfactory cues had properly been eliminated. One observer, who was unaware of the rats'

group assignment, recorded the data manually without TV monitoring.

2.5. Statistics

Overall analyses were carried out with one-way or two-way analysis of variance (ANOVA). Group comparisons were made with Newman–Keuls post hoc test. Computations were made with Prism statistical software program (GraphPad Software, CA, USA).

3. Results

3.1. Experiment 1

In Tables 1 and 2, significant differences relative to the saline group are presented. Only significant differences among the various treatment groups are presented in this section. In Session I, both the scopolamine and procyclidine groups displayed significantly lower preference for novelty than the HI-6 and levetiracetam groups ($P < 0.05$). In Sessions II and III, both the scopolamine and procyclidine groups displayed significantly lower preference for novelty than the HI-6, physostigmine, and levetiracetam groups ($P < 0.05$). The total time exploring objects did not differ significantly among the groups for Phases 1 and 2 in Session I ($F(5,42) = 2.184, P > 0.05$; $F(5,42) = 2.292, P > 0.05$, respectively). In Phase 1 in Session II, the scopolamine group explored the novel object reliably less than the HI-6 and physostigmine groups ($P < 0.05$). In Phase 2 in Session II, the scopolamine group had significant lower exploring time than the HI-6, physostigmine, and levetiracetam groups ($P < 0.05$) and the procyclidine group had reliably lower exploring time than the HI-6 and levetiracetam groups ($P < 0.05$). In Phases 1 and 2 in Session III, the scopolamine and procyclidine groups displayed significantly less exploring than the HI-6, physostigmine, and levetiracetam groups ($P < 0.05$).

As seen from Table 2, rats treated with scopolamine, physostigmine, or procyclidine tended to display less motor activity than the control animals. Two-way ANOVA revealed a significant Group \times Time (Session/Phase) interaction ($F(25,252) = 4.784, P = 0.0001$), a significant between group factor ($F(5,50) = 43.29, P < 0.0001$), as well as a significant within group factor ($F(5,252) = 20.55, P < 0.0001$). In Phase 2 in Session I, the physostigmine and procyclidine groups were significantly less active than the HI-6 and levetiracetam groups ($P < 0.01$). In Phase 1 in Session II, the scopolamine, physostigmine, and procyclidine groups were reliably less active than the HI-6 and levetiracetam groups ($P < 0.01$). In Phase 2 in Session II, the procyclidine group displayed significantly less motor activity than the HI-6, physostigmine, and levetiracetam groups ($P < 0.01$). In Phase 1 in Session III, the scopolamine, physostigmine, and procyclidine groups were reliably less active than the HI-6 and levetiracetam groups ($P < 0.001$). In Phase 2 in Session III, the scopolamine and procyclidine groups were reliably less active than the HI-6 and levetiracetam groups ($P < 0.01$) and the physostigmine group was significantly less active than the levetiracetam group ($P < 0.05$).

Table 2 Mean (\pm SEM) measures of locomotor (squares) and rearing activity in novelty test in Experiment 1.

Group	Squares Session						Rearings Session					
	I		II		III		I		II		III	
	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2
Saline	88.9 \pm 6.5	61.5 \pm 9.5	84.1 \pm 5.6	72.3 \pm 8.1	89.3 \pm 9.5	66.0 \pm 5.9	21.5 \pm 1.5	14.3 \pm 2.6	25.5 \pm 2.2	19.1 \pm 1.7	23.9 \pm 3.2	18.1 \pm 2.7
HI-6	89.8 \pm 6.8	58.6 \pm 7.9	90.1 \pm 2.9	56.4 \pm 5.8	86.0 \pm 6.1	52.9 \pm 6.0	21.9 \pm 2.6	17.4 \pm 3.0	24.8 \pm 2.5	17.9 \pm 1.6	22.9 \pm 2.3	17.1 \pm 1.7
Scopolamine	83.4 \pm 5.9	42.3 \pm 9.8	45.7 \pm 9.3 ^b	38.9 \pm 8.6 ^b	25.3 \pm 9.8 ^c	18.4 \pm 8.9 ^c	22.7 \pm 2.8	19.3 \pm 1.9	17.4 \pm 2.4 ^a	15.3 \pm 1.9	18.5 \pm 3.3	14.7 \pm 1.8
Physostigmine	62.5 \pm 6.1	22.3 \pm 1.9 ^b	60.9 \pm 6.1 ^a	53.8 \pm 9.0	39.8 \pm 9.1 ^c	37.5 \pm 6.7 ^a	15.3 \pm 1.8	9.6 \pm 2.1	14.8 \pm 3.0 ^a	12.9 \pm 3.1	8.1 \pm 1.4 ^c	11.3 \pm 1.7
Levetiracetam	82.1 \pm 7.7	63.9 \pm 9.6	98.8 \pm 7.6	68.0 \pm 10.1	80.9 \pm 4.6	60.9 \pm 7.7	21.4 \pm 1.5	14.8 \pm 2.3	27.8 \pm 1.9	20.1 \pm 2.5	23.9 \pm 3.1	20.8 \pm 2.6
Procyclidine	68.2 \pm 8.7	18.3 \pm 3.1 ^b	21.8 \pm 3.7 ^c	17.9 \pm 4.4 ^c	16.8 \pm 3.8 ^c	15.3 \pm 3.3 ^c	12.7 \pm 2.3 ^a	3.9 \pm 1.9 ^b	3.5 \pm 1.8 ^c	4.8 \pm 2.1 ^c	4.3 \pm 2.1 ^c	4.5 \pm 2.2 ^c

Doses and number of animals as in Table 1. Significantly different from the saline group:

^a $p < 0.05$,

^b $p < 0.01$,

^c $p < 0.001$.

Table 3

Mean (\pm SEM) measures of exploratory behavior in seconds in novelty test in Experiment 2. Differential time exploring is the difference between exploring novel and neutral items.

Group	N	Dose mg/kg	Differential time exploring			Total time exploring					
			Session			Session					
			I Phase 2	II Phase 2	III Phase 2	I Phase 1	II Phase 1		II Phase 2		III Phase 1
Saline	8	----	7.8 \pm 3.1	21.6 \pm 3.3	13.8 \pm 2.2	18.8 \pm 2.9	16.5 \pm 2.9	14.7 \pm 2.5	29.8 \pm 6.3	15.4 \pm 2.0	23.0 \pm 1.9
HI-6	8	125	0.7 \pm 1.2 ^a	2.9 \pm 1.8 ^c	1.5 \pm 0.9 ^c	8.3 \pm 2.4 ^a	3.8 \pm 1.1 ^b	3.5 \pm 2.0 ^a	6.8 \pm 1.8 ^b	0.7 \pm 0.9 ^c	4.2 \pm 0.8 ^c
Scopolamine		1									
Physostigmine		0.1									
HI-6	8	125	1.5 \pm 0.9 ^a	4.2 \pm 1.4 ^c	2.4 \pm 0.5 ^c	14.9 \pm 2.8	8.3 \pm 3.1 ^a	11.0 \pm 3.5	8.5 \pm 1.7 ^c	2.5 \pm 1.6 ^c	4.9 \pm 1.0 ^c
Levetiracetam		50									
Procyclidine		20									

Significantly different from the saline group:

^a $p < 0.05$,

^b $p < 0.01$,

^c $p < 0.001$.

Table 4 Mean (\pm SEM) measures of locomotor (squares) and rearing activity in novelty test in Experiment 2.

Group	Squares						Rearings					
	I		II		III		I		II		III	
	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2
Saline	98.5 \pm 10.5	71.1 \pm 9.3	87.3 \pm 11.6	63.8 \pm 9.6	75.8 \pm 10.1	68.9 \pm 12.3	25.1 \pm 1.7	18.5 \pm 2.8	24.7 \pm 2.8	21.0 \pm 3.0	26.8 \pm 3.4	19.8 \pm 2.7
HI-6												
Scopolamine	53.3 \pm 7.3 ^a	22.3 \pm 6.2 ^b	34.8 \pm 3.3 ^b	19.5 \pm 3.0 ^b	23.7 \pm 2.9 ^b	17.5 \pm 3.3 ^b	16.4 \pm 1.8 ^a	13.8 \pm 2.1	18.4 \pm 3.7	8.9 \pm 2.5 ^a	7.9 \pm 2.6 ^b	6.8 \pm 1.8 ^b
Physostigmine												
HI-6												
Levetiracetam	60.3 \pm 9.9 ^a	15.4 \pm 3.4 ^b	21.6 \pm 4.0 ^b	16.6 \pm 3.3 ^b	14.8 \pm 1.9 ^b	11.0 \pm 2.5 ^b	11.8 \pm 2.5 ^b	2.6 \pm 1.5 ^b	2.9 \pm 1.4 ^b	3.5 \pm 1.4 ^b	1.4 \pm 0.5 ^b	3.1 \pm 1.4 ^b
Procyclidine												

Doses and number of animals as in Table 3. Significantly different from the saline group:

^a $p < 0.01$,
^b $p < 0.001$.

The rearing activity also differed among the groups (Table 2). Two-way ANOVA revealed a significant between group factor ($F(5,50) = 44.10, P < 0.0001$), as well as a significant within group factor ($F(5,252) = 6.955, P < 0.0001$), but not a Group \times Time (Session/Phase) interaction ($F(25,252) = 1.423, P = 0.0924$). In Phase 1 in Session I, the procyclidine group made significantly less rearing than the HI-6 and levetiracetam groups ($P < 0.05$). In Phase 2 in Session I, the procyclidine group made reliably less rearing than the HI-6, scopolamine, and levetiracetam groups ($P < 0.01$). In Phase 1 in Session II, the procyclidine group made less rearing than the HI-6, scopolamine, physostigmine, and levetiracetam groups ($P < 0.01$). The scopolamine and physostigmine groups made significantly less rearing than the HI-6 and levetiracetam groups ($P < 0.05$). In Phase 2 in Session II, the procyclidine group made less rearing than the HI-6, scopolamine, physostigmine, and levetiracetam groups ($P < 0.05$). In Phase 1 in Session III, the procyclidine group made less rearing than the HI-6, scopolamine, and levetiracetam groups ($P < 0.01$). The physostigmine group made less rearing than the HI-6, scopolamine, and levetiracetam groups ($P < 0.01$). In Phase 2 in Session III, the procyclidine group made less rearing than the HI-6, scopolamine, and levetiracetam groups ($P < 0.05$).

3.2. Experiment 2

All significant differences found between the treatments are presented in Tables 3 and 4. Both the physostigmine and procyclidine regimens caused decreased locomotor activity (Table 4). Two-way ANOVA revealed a significant between group factor ($F(2,25) = 101.2, P < 0.0001$), as well as a significant within group factor ($F(5,126) = 12.07, P < 0.0001$), but not a Group \times Time (Session/Phase) interaction ($F(10,126) = 0.4996, P > 0.05$). The physostigmine and procyclidine regimens also reduced the number of rearings (Table 4). Two-way ANOVA revealed a significant Group \times Time (Session/Phase) interaction ($F(10,126) = 2.048, P = 0.0336$), a significant between group factor ($F(2,25) = 94.00, P < 0.0001$), as well as a significant within group factor ($F(5,126) = 4.838, P = 0.0004$).

3.3. Experiment 3

Decreased preference for novelty was seen in several groups (Table 5). In Session II, both the DCG-IV and MPEP regimens displayed less preference than DCG-IV ($P < 0.05$). In Session III, both treatment regimens caused less preference for novelty than the MPEP ($P < 0.01$). The total time exploring differed somewhat among the groups. In Phase 2 in Session III, the DCG-IV regimen caused less exploring than MPEP ($P < 0.05$).

Reduced locomotor activity was seen among the groups (Table 6). Two-way ANOVA revealed a significant between group factor ($F(4,42) = 38.62, P < 0.0001$), as well as a significant within group factor ($F(5,210) = 7.717, P < 0.0001$), but not a Group \times Time (Session/Phase) interaction ($F(20,210) = 1.006, P > 0.05$). Some significant differences between the treatment groups were found. In Phase 1 in Session II, the DCG-IV regimen produced less locomotion than DCG-IV and MPEP ($P < 0.05$). The MPEP regimen

Table 5

Mean (\pm SEM) measures of exploratory behavior in seconds in novelty test in Experiment 3. Differential time exploring is the difference between exploring novel and neutral items.

Group	N	Dose mg/kg	Differential time exploring			Total time exploring					
			Session			Session					
			I	II	III	I	II	III	I	II	III
Saline	8	---	Phase 2 7.2 \pm 2.1	Phase 2 23.2 \pm 6.2	Phase 2 12.5 \pm 2.5	Phase 1 18.5 \pm 2.7	Phase 2 15.3 \pm 2.9	Phase 1 10.9 \pm 2.0	Phase 2 29.9 \pm 6.7	Phase 1 13.4 \pm 2.8	Phase 2 21.8 \pm 2.4
DCG-IV	8	4	3.8 \pm 2.1	18.8 \pm 3.0	6.4 \pm 1.1	13.3 \pm 2.5	12.6 \pm 3.7	8.4 \pm 0.7	21.0 \pm 3.6	11.0 \pm 1.8	13.1 \pm 2.6
MPEP	8	30	9.5 \pm 2.6	10.9 \pm 2.4 ^a	12.3 \pm 2.7	21.0 \pm 3.2	19.0 \pm 4.4	9.9 \pm 1.2	17.9 \pm 3.0	8.0 \pm 1.8	17.0 \pm 2.9
HI-6	8	125	2.9 \pm 1.1	3.9 \pm 1.7 ^b	3.0 \pm 0.8 ^b	16.3 \pm 3.6	10.0 \pm 4.5	9.8 \pm 2.3	9.3 \pm 1.9 ^b	5.8 \pm 2.4	6.1 \pm 2.7 ^c
Procyclidine		20									
DCG-IV		4									
HI-6	8	125	3.4 \pm 1.1	2.8 \pm 0.7 ^b	2.7 \pm 1.4 ^b	13.8 \pm 2.4	13.1 \pm 4.2	7.9 \pm 1.8	7.3 \pm 2.0 ^b	3.0 \pm 1.3	9.6 \pm 2.0 ^b
Procyclidine		20									
MPEP		30									

Significantly different from the saline group:

^a $p < 0.05$,

^b $p < 0.01$,

^c $p < 0.001$.

Table 6
Mean (\pm SEM) measures of locomotor (squares) and rearing activity in novelty test in Experiment 3.

Group	Squares						Rearings					
	I		II		III		I		II		III	
	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2	Phase 1	Phase 2
Saline	89.3 \pm 9.1	76.7 \pm 8.5	91.3 \pm 11.1	74.8 \pm 10.3	86.8 \pm 9.9	73.4 \pm 9.6	24.3 \pm 3.7	17.9 \pm 2.9	25.3 \pm 1.6	19.8 \pm 2.5	26.1 \pm 2.9	17.8 \pm 2.3
DCG-IV	79.0 \pm 7.9	46.3 \pm 14.2 ^a	88.0 \pm 13.0	66.6 \pm 11.0	56.8 \pm 9.0 ^a	42.4 \pm 8.6 ^b	22.6 \pm 3.0	12.5 \pm 3.4	22.9 \pm 4.0	19.1 \pm 1.9	17.1 \pm 3.2	14.8 \pm 3.1
MPEP	64.8 \pm 11.1	42.5 \pm 10.5 ^a	58.3 \pm 10.7	49.8 \pm 8.0	54.1 \pm 12.5 ^a	41.9 \pm 7.5 ^b	17.5 \pm 2.9	13.3 \pm 2.7	16.4 \pm 3.0 ^a	14.1 \pm 2.6	18.9 \pm 3.4	17.6 \pm 2.4
HI-6	56.7 \pm 10.2	21.4 \pm 4.8 ^b	18.7 \pm 3.8 ^c	15.2 \pm 3.1 ^c	16.4 \pm 1.8 ^c	13.9 \pm 2.2 ^c	12.4 \pm 2.7	8.7 \pm 1.8	5.9 \pm 0.8 ^c	6.1 \pm 2.3 ^c	5.7 \pm 1.8 ^c	3.8 \pm 1.0 ^b
Procyclidine												
DCG-IV	63.6 \pm 14.3	38.7 \pm 8.5 ^a	32.1 \pm 6.7 ^c	23.9 \pm 4.2 ^c	28.3 \pm 5.1 ^c	25.8 \pm 3.4 ^c	13.9 \pm 2.2	10.4 \pm 2.8	6.1 \pm 1.8 ^c	5.8 \pm 1.3 ^c	10.1 \pm 0.9 ^c	6.7 \pm 2.2 ^b
HI-6												
Procyclidine												
MPEP												

Doses and number of animals as in Table 5. Significantly different from the saline group:

^a $p < 0.05$,

^b $p < 0.01$,

^c $p < 0.001$.

caused less locomotion than DCG-IV ($P < 0.001$). The DCG-IV and MPEP regimens resulted in less locomotor activity than DCG-IV and MPEP ($P < 0.05$). In Phase 1 in Session III, the DCG-IV regimen produced less locomotion DCG-IV and MPEP ($P < 0.05$). In Phase 2 in Session III, the DCG-IV regimen produced less locomotion DCG-IV and MPEP ($P < 0.05$).

The rearing activity also differed among the groups (Table 6). Two-way ANOVA revealed a significant between group factor ($F(4,42) = 35.98, P < 0.0001$), as well as a significant within group factor ($F(5,210) = 4.061, P = 0.0015$), but not a Group \times Time (Session/Phase) interaction ($F(20,210) = 1.115, P > 0.05$). In Phase 1 in Session II, the DCG-IV and MPEP regimens resulted in less rearing activity than DCG-IV and MPEP ($P < 0.05$). In Phase 2 in Session II, the DCG-IV and MPEP regimens caused less rearing activity than DCG-IV and MPEP ($P < 0.05$). In Phase 1 in Session III, the DCG-IV regimen produced less rearing than DCG-IV and MPEP ($P < 0.05$). In Phase 1 in Session III, the DCG-IV and MPEP regimens resulted in less rearing activity than DCG-IV and MPEP ($P < 0.05$).

4. Discussion

The results from the present study clearly showed that the prophylactic regimens tested can have marked cognitive side effects. The preference for novelty was abolished by the physostigmine and procyclidine regimens and reduced by the DCG-IV and MPEP regimens (Table 7). Scopolamine and procyclidine separately impaired preference for novelty pronouncedly, whereas MPEP exerted a slight adverse effect. HI-6, physostigmine, levetiracetam, and DCG-IV alone did not cause cognitive deficits. Locomotor and rearing activities did not differ much between the various regimens, but large differences were seen between the single drugs.

Some relations between the degree of cognitive performance and prophylactic capacities can be seen for the regimens. The most efficient protection is obtained by the physostigmine and procyclidine regimens, whereas the DCG-IV and MPEP regimens have weaker prophylactic capacities [11,12]. The total time exploring, reflecting the interest in the environment, was less reduced in the rats given the DCG-IV or MPEP regimens than the rats that received the physostigmine or procyclidine regimens. On the other hand, all 4 regimens made the rats very inactive as a marked decrement in locomotor and rearing activities was measured.

When combining a cholinesterase inhibitor like physostigmine and an anticholinergic like scopolamine equalizing effects are expected to occur [3]. In view of the present results, this was not the case for cognitive performance or activity measures. A potential explanation of the adverse effects might be that continuous stimulation of acetylcholine receptors resulted in desensitization and enhanced efficacy of anticholinergic drugs. If procyclidine is used in lower doses (1 and 3 mg/kg) in combination with physostigmine (0.1 mg/kg), much milder interference with behavioral functions are seen in the present novelty task [25].

Scopolamine has indisputable detrimental effect on the performance of rats in water maze, radial maze, passive

avoidance, and spontaneous alternation [26]. In behavioral studies, the dose of scopolamine commonly used is 0.5 mg/kg [27]. However, scopolamine has been reported to impair performance in delayed matching to position tasks at doses as low as 0.05 and 0.075 mg/kg in rats [28,29]. It has been reported that general locomotor activity can be depressed following administration of acetylcholinesterase inhibitors such as physostigmine [30]. In contrast, classical acetylcholine receptor antagonists like scopolamine and atropine generally increase measures of activity in many species, including the rat [31,32]. In the present study, scopolamine and physostigmine tended to reduce both locomotor and rearing activity.

HI-6 and levetiracetam had no negative effects on any of the behavioral measures used. Procyclidine at a dose of 20 mg/kg impaired all aspects of behavior in the novelty test. Hence, the pronounced impairment of the procyclidine regimen is most likely attributable to the relatively high dose of procyclidine that is required for the procyclidine regimen to become effective against soman-induced seizures [11]. Procyclidine at a dose of 3 mg/kg is without effects in the novelty test, whereas 6 mg/kg has adverse effects [25]. Relatively low doses of procyclidine (1, 3, 6 mg/kg) are sufficient when this drug is combined with physostigmine (0.1 mg/kg) to protect against convulsant doses of soman (1.3 \times , 1.6 \times , 2.0 \times LD₅₀, respectively) [5].

HI-6 (125 mg/kg) did not produce any adverse behavioral effects in the present test. This finding corresponds well with previous results from rats. Only doses above 125 mg/kg of HI-6 have adverse effects on open field activity, motor coordination, or shuttle-box performance [33]. HI-6 at the highest dose used (100 mg/kg) disrupts operant fixed ratio responding, but not performances in shuttle-box, drinking motivation, exploratory behavior, negative geotaxis, or suspension time [34]. Furthermore, HI-6 at a dose above 154 mg/kg results in impairment of an operant task using a variable-interval 56 s schedule of food reinforcement [35].

Levetiracetam (50 mg/kg) had no effect on the parameters used in the novelty task. Absence of negative impact on behavior has been reported for even high doses of levetiracetam in rats. Levetiracetam up to 170 mg/kg does not impair the performance in water maze [36]. Levetiracetam at doses up to 200 mg/kg have no detrimental efficacy on operant five-choice serial reaction task or operant repeated acquisition of response sequences task [37,38].

A high dose of procyclidine (20 mg/kg) can affect several aspects of behavior as seen from the present study. Procyclidine in a dose range of 0.1–5.6 does not impair acoustic startle response in rats [39]. A dose of 1 mg/kg of procyclidine does not attenuate performance in an operant conditioning task, whereas impairment is seen after a dose of 10 mg/kg [40].

To our knowledge, no study has examined behavioral effects of systemic administration of DCG-IV in rats. There is, however, one study of mice showing that DCG-IV (5 or 10 mg/kg) reduces locomotor activity in a test apparatus [41]. This finding coincides with our results that the only impact of DCG-IV (4 mg/kg) was a moderate decline in locomotion.

Table 7
Preference for novelty relative to saline-treated rats.

Experiment 1		Session		
Group	mg/kg	I	II	III
HI-6	125	—	—	—
Scopolamine	1	↓	↓	↓
Physostigmine	0.1	—	—	—
Levetiracetam	50	—	—	—
Procyclidine	20	↓	↓	↓
Experiment 2		Session		
Group	mg/kg	I	II	III
HI-6	125	↓	↓	↓
Scopolamine	1			
Physostigmine	0.1			
HI-6	125	↓	↓	↓
Levetiracetam	50			
Procyclidine	20			
Experiment 3		Session		
Group	mg/kg	I	II	III
DCG-IV	20	—	—	—
MPEP	30	—	↓	—
HI-6	125	—	↓	↓
Procyclidine	20			
DCG-IV	4			
HI-6	125	—	↓	↓
Procyclidine	20			
MPEP	30			

↓, decreased; —, unchanged.

MPEP (10 mg/kg) has been shown to impair response rate, but not response accuracy in operant repeated acquisition, whereas spatial memory in radial maze is not affected [42]. MPEP at 3 or 10 mg/kg does not interfere with locomotor activity, whereas only the highest dose decreases performance in spontaneous alternation and operant learning [43]. Furthermore, MPEP (3, 6, or 12 mg/kg) causes detrimental effects on latent inhibition in conditioned taste aversion in a dose-dependent manner [44]. However, MPEP at a dose of 30 mg/kg does not affect rotarod locomotor performance in rats [45]. Hence, it was not surprising that our relatively high MPEP dose (30 mg/kg) produced a moderate cognitive deficit and also affected locomotor activity and barely influenced rearing activity.

Of the single drugs tested, scopolamine and procyclidine produced severe cognitive deficits. Scopolamine attenuates normal cholinergic activity that is a prerequisite for normal attention processes [46]. Since the scopolamine group also tended to display less total time exploring, these rats might have paid less attention to the surroundings as well as the objects. The rats that received procyclidine behaved in a similar way showing both cognitive deficit and reduced total time exploring. In addition to cholinergic antagonism, procyclidine also exerts NMDA antagonism [47,48]. Thus, impairment of working memory (contemporary comparing objects) as well as reference memory (preserving information from Phase 1 to Phase 2) may have contributed to the present results. It cannot be excluded, however, that a non-cognitive factor like motor dysfunction may be associated with the behavioral changes.

In order to ensure complete protection against a convulsant dose of soman, comprehensive interference with the central nervous system is required. Thus, from a theoretical point of view, behavioral side effects will likely occur. The physostigmine and procyclidine regimens that produce complete protection against lethal doses of soman [11] caused the most severe side effects in the present study. If partial protection followed by adjunct treatment after exposure to nerve agent is acceptable, pretreatment with HI-6 and levetiracetam accompanied by procyclidine might be an option. HI-6 and levetiracetam were without behavioral side effects in the present study. The potent anticonvulsant procyclidine (anticholinergic and antiglutamatergic) is more appropriately used as a post-poisoning drug than as a prophylactic agent. The potency of HI-6 and levetiracetam used as prophylactics followed by procyclidine as adjunct has partly been tested before. Rats pretreated with HI-6 that were about to die 5–10 min after onset of soman-induced seizures survived and recovered well when they were treated with levetiracetam and procyclidine [21].

In conclusion, prophylactic regimens previously demonstrated to effectively protect against lethal doses of soman exert adverse effects on behavior. To circumvent the problem drugs with antidotal efficacy, but without behavioral side effects (HI-6, levetiracetam) may be used as prophylactics followed by a more powerful adjunct therapy (procyclidine) after challenge with nerve agent.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.toxrep.2014.04.004](https://doi.org/10.1016/j.toxrep.2014.04.004).

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