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Beneficial effects of galantamine on performance in the object recognition task in Swiss mice: Deficits induced by scopolamine and by prolonging the retention interval

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Abstract

Episodic memory has been found to be impaired in several neuropsychiatric disorders. The object recognition task (ORT), introduced by Ennaceur and Delacour [Ennaceur A., Delacour J. A new one-trial test for neurobiological studies of memory in rats: 1. Behavioral data. *Behav Brain Res* 1988; 31: 47–59.], is a method to measure a specific form of episodic memory in rats and mice. It is based on the spontaneous behavior of rodents and can be considered as a retention test completely free of reference memory components. Therefore, the ORT has been increasingly used as an experimental tool in assessing drug effects on memory and investigating the neural mechanisms underlying learning and memory.

In the present study, the main goal was to evaluate the effects of galantamine in Swiss mice in the ORT on scopolamine-induced deficits and with different retention intervals. Mice had a good object recognition memory at the 15 min retention intertrial interval (ITI). Object discrimination was absent at the longer intervals (1 h, 4 h and 24 h). Galantamine (10 mg/kg, administered s.c., 30 min prior to acquisition) partially reversed effects of scopolamine (0.63 mg/kg, administered s.c., 30 min prior to acquisition) and normalized performance to control levels. A lower dose of galantamine (0.63 mg/kg) was also investigated when two different retention intervals (15 min and 1 h) were used. Galantamine (0.63 mg/kg) had no adverse effects. Solvent-treated mice in the 1 h ITI condition did not discriminate between the novel and the familiar object (discrimination index was equal to zero), while galantamine (0.63 mg/kg)-treated mice attained a good object recognition memory performance.

In conclusion, galantamine was shown to possess memory-enhancing effects in two conditions that reduced object discrimination: scopolamine-induced deficits and when a longer retention interval was used.

Keywords: Object recognition task (ORT); Galantamine; Scopolamine; Swiss mice; Retention interval

1. Introduction

The initial phase of Alzheimer's disease (AD) is marked by a progressive deterioration of episodic memory (Lindeboom and Weinstein, 2004; Riepe, 2005). Also, in bipolar disorder (BPD), episodic memory has been considered to be one of the most consistent areas of impairment (Osuji and Cullum, 2005). Finally, several studies have indicated that episodic memory deficits are also particularly prominent in schizophrenic patients, and are observed even in untreated patients in their first episode of the disorder (Aleman et al., 1999; Flashman and Green, 2004; Heckers et al., 2000; Heinrichs and Zakzanis, 1998).

The object recognition task (ORT), introduced by Ennaceur and Delacour (1988), is a method to measure a specific form of episodic memory in rats and mice. It is based on the spontaneous behavior of rodents and can be considered as a retention test completely free of reference memory components, such as rule learning. Moreover, it is not based on usual positive or negative reinforcers such as food or electric shocks, which make the interpretation of drug effects on memory difficult. Therefore, the ORT has been increasingly used as an experimental tool in assessing drug effects on memory and investigating the neural mechanisms underlying learning and memory (De Lima et al., 2005).

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The ORT is based on the differences in exploring familiar as opposed to unfamiliar objects. Rodents are first exposed to two identical objects. After a predetermined retention delay, one of the objects presented in the first trial is replaced by a new one. The ability of rodents to distinguish between the two objects in the second trial is revealed by the animal spending more time exploring the new object than the familiar one. Differences in exploration time constitute the discrimination index that is used as parameter.

The ORT has been predominantly used as a preclinical test to investigate the memory-enhancing effects of acetylcholinesterase inhibitors (AChEIs) such as donepezil, used as treatments for AD. These drugs presumably act by raising and prolonging the profile of acetylcholine (ACh) via an inhibitory effect on the esterase. For example, Prickaerts et al. (2005) have studied the effects of donepezil and metrifonate (administered orally; 30 min before or immediately after learning to study acquisition and consolidation, respectively). Based on their results, the authors have concluded that AChEIs improve processes of acquisition of object information rather than consolidation processes.

Galantamine (Reminyl), a plant alkaloid, also belongs to this class of AChEIs. This drug has been approved for symptomatic treatment of vascular dementia and AD. Currently, there is also a clinical study ongoing to examine whether adjunctive galantamine is effective in the treatment of cognitive impairments in patients with schizophrenia (University of Maryland Baltimore School of Medicine, Maryland Psychiatric Research Center).

In contrast to donepezil, galantamine has been considered to be a rather weak AChEI (Geerts and Guillaumat et al., 2005). Besides acting on acetylcholinesterase, galantamine has been suggested to have several other mechanisms of action. Galantamine has been proposed to allosterically modulate nicotinic acetylcholine receptors (Albuquerque et al., 1997; Samochocki et al., 2003); potentiate activity of *N*-methyl-D-aspartate (NMDA) receptors (Moriguchi et al., 2004) and possess neuroprotective effects (Arias et al., 2004; Kihara et al., 2004; Sobrado et al., 2004). Galantamine (1.25–5.0 mg/kg, i.p.) has been shown to be effective in improving cognition in several preclinical tests in rodent models exhibiting cognitive deficits. For example, galantamine has been found to attenuate scopolamine-induced deficits in the T-maze reinforced alternation procedure, Morris water maze and in a passive avoidance paradigm in rats (Bores et al., 1996; Chopin and Briley, 1992; Fishkin and Ince et al., 1993). In addition, sub-chronic administration of galantamine has been reported to reduce deficits in the Morris water maze in APP23 mice, a potential model of AD (Van Dam et al., 2005). Finally, Iliev et al. (2000) have examined the effect of galantamine on learning ability using the shuttle-box test, after a 20-min carotid artery occlusion in rats. Galantamine was shown to have a beneficial effect on the recovery of learning ability post-ischaemia.

In the present study, our goal was to investigate the effects of galantamine in mice in the ORT on scopolamine-induced deficits and with different retention intervals. The purpose was to find out whether galantamine could be used as a positive control in future drug studies evaluating potential novel cognitive-enhancing compounds. We performed four experiments. In the first study, the effect of different retention intervals between trials 1 and 2 (intertrial interval or ITI of 15 min, 1, 4 or 24 h) on novel object exploration were investigated in Swiss-SPF (CD1) Crl:CD1(Icr) mice. The aim was to establish the ITI at which optimal and reduced performance occurred. Based on the results, it was concluded that good performance was obtained at the 15 min ITI. In the second experiment, the dose of scopolamine was established at which deficits were found in the ORT. Deficits were most obvious when 0.63 mg/kg of scopolamine was administered subcutaneously (s.c.) 30 min prior to trial 1 (T-30). Third, potential reversal with galantamine (0.63, 2.5 and 10 mg/kg, s.c., T-30) of scopolamine-induced deficits was tested. Fourth, effects of the lowest dose of galantamine (0.63 mg/kg) were investigated when two different retention intervals were used: the ITI of 15 min with very good performance and the 1 h ITI at which sub-optimal object recognition memory occurred.

2. Materials and methods

2.1. Animals

Male Swiss-SPF (CD1) Crl:CD1(Icr) mice, obtained from Charles River (Sulzfeld, Germany), 172 animals in total, were used. For each experiment, mice were only used once and each experimental group consisted of 10–12 animals. Mice were 12–13 weeks old and approximately 30 g in weight at the time of the experiment.

All animals were housed in their individual home cages, at least 3 days prior to testing under controlled conditions (temperature: 23 °C, humidity: 60%, normal light–dark cycle: light on 06:00 until 18:00). Animals were provided with a supply of food and water ad libitum. All efforts were made to minimize animal discomfort and for limiting the numbers of animals used. The experiments were carried out following the procedure described by the guidelines of the European Community Council Directive of 24 November 1986 (Declaration of Helsinki 86/609/EEC).

2.2. Apparatus

The apparatus consisted of a transparent polyvinyl chloride circular arena, 45 cm in diameter. In each trial, two objects were placed in a symmetrical position about 5 cm away from the wall. We used two different sets of objects: a cone made of brass with a hole in the middle and an aluminium cone with a tapering top (both 6 cm in diameter). A mouse could not displace the objects. The order of objects used per subject per session was determined randomly. All combinations and locations of objects were used in a balanced manner to reduce potential biases due to preferences for particular locations or objects. Also, in a previous inhouse experiment, it was found that the mice showed no preference for a certain object or location (data not shown). In addition, a computerized tracking system and image analyzer (EthoVision® 3.0.15, Noldus, Wageningen, The Netherlands) was used to monitor walking patterns. The camera hung perpendicular to the center of the setup. The image analyzer tracked the center of each mouse

with a sampling rate of 25 Hz, and allowed the calculation of distance traveled (in cm).

2.3. Procedures

The protocol according to Sik et al. (2003) was adjusted. The day before the actual test, the animals were adapted to the test box, i.e. they were allowed to explore the apparatus (without any objects) for 3 min. Subsequently, the mice underwent the testing session comprised of two trials. The duration of each trial was 3 min. During the first trial (T1, *sample phase*), the apparatus contained two identical objects (samples). A mouse was always placed in the apparatus facing the wall. After the first exploration period, the mouse was put back in its home cage. Subsequently, after a predetermined retention interval (intertrial interval or ITI of 15 min, 1, 4 or 24 h), the mouse was placed in the apparatus for the second trial (T2, *choice phase*), but now with two dissimilar objects, a familiar one (the sample) and a new one. The objects were always cleaned between sessions. The times spend in exploring each object during T1 and T2 were recorded using the manual tracking option of the EthoVision system. Exploration was defined as "touching the object with the nose". Measures involved in the object recognition test were the following: $e_1 = a_1 + a_2$; $e_2 = a + b$; $d_1 = b - a$; $d_2 = d_1/e_2$, where e_1 is the time spend in exploring both identical objects (a_1 and a_2) in the first trial; e_2 is the time spend in exploring both the familiar (a) and new (b) object in the second trial; d_1 (absolute difference) and d_2 (relative) are the measures of discrimination between the new and familiar objects. The difference in exploration time on T1 and T2 (e_1 and e_2) can be considered as habituation measure. Animals with a total exploration time of less than 7 s during T1 or T2 were discarded from the analyses. In house tests with other strains such as the C57BL/6J showed that the recognition index could not be reliably measured in these animals, since they showed exploration levels below 7 s (data not shown). In addition, Swiss mice in the present experiments were also required to spend time exploring both identical objects during T1 in order to be included in the analyses. This meant that of the 43 control Swiss mice tested under the 15 min ITI condition in the four experiments, only 3 animals were discarded from the analyses in the present study.

2.4. Experiments

(1) Effects of different retention intervals between T1 and T2 on novel object exploration were measured in 40 Swiss mice (ITI of 15 min, 1, 4 or 24 h).
(2) Effects of scopolamine (0.04, 0.16 and 0.63 mg/kg, s.c., T-30 min) on novel object exploration were measured in 40 Swiss mice (ITI set at 15 min).
(3) Reversal of scopolamine (0.63 mg/kg, s.c., T-30)-induced novel object exploration deficits by galantamine (0.63, 2.5 and 10 mg/kg, s.c., T-30) were measured in 52 Swiss mice (ITI set at 15 min).
(4) Effects of galantamine (0.63 mg/kg, s.c., T-30) on novel object exploration with different retention intervals (15 min and 1 h) were measured in 40 Swiss mice.

2.5. Drug treatments

In the scopolamine dose-response study, either saline or scopolamine (0.04, 0.16 and 0.63 mg/kg, s.c.) was injected 30 min prior to T1. In the second drug study, both scopolamine (0.63 mg/kg, s.c.) and galantamine (0.63, 2.5 and 10 mg/kg, s.c.) were injected 30 min prior to the acquisition session (T1). The dose of scopolamine (0.63 mg/kg) was based on the findings from the dose-response study: results were the most robust. At the 0.16 mg/kg dose, d_2 tended to differ from zero. Furthermore, at the 0.63 mg/kg dose, none of the mice had a d_2 higher than 0.3. Also, in another study in Swiss mice, even relatively higher doses (0.3, 1 and 3 mg/kg, s.c.) were used to disrupt performance (Dodart et al., 1997). In the final study, the lowest dose of galantamine (0.63 mg/kg, s.c.) was injected 30 min prior to T1. (−)-Scopolamine hydrobromide was purchased from Sigma Aldrich (Bornem, Belgium). Galantamine hydrobromide was obtained from Janssen Pharmaceutica (Beerse, Belgium). All drugs were dissolved in saline.

2.6. Statistical analyses

The basic measures were the times spend by the mice in exploring an object during T1 and T2. d_2 was considered as the index measure of discrimination between the new and the familiar objects. In fact, d_2 is a relative measure of discrimination that corrects d_1 for exploration activity (e_2).

Table 1
Discrimination index (d2) for each experimental group — difference from zero: *t*, *p* values and degrees of freedom (*df*) from One-Sample Test

Experiment	Groups (doses are indicated between brackets, in mg/kg)	*n*	One-Sample Test (Test Value for $d_2=0$)		
			t	*df*	*p*
(A) ITI	15 min ITI	10	7.83	9	<0.001***
	1 h ITI	10	0.63	9	n.s.
	4 h ITI	8	0.67	7	n.s.
	24 h ITI	8	1.86	7	n.s.
(B) Scopolamine dose–response	Solvent	8	6.42	7	<0.001***
	Scop(0.04)	9	1.54	8	n.s.
	Scop(0.16)	10	1.85	9	=0.097(*)
	Scop(0.63)	8	−0.60	7	n.s.
(C) Galantamine/ Scopolamine	Solvent/Solvent	11	10.93	10	<0.001***
	Solvent/Scop(0.63)	11	0.24	10	n.s.
	Gal(0.63)/Scop(0.63)	8	1.42	7	n.s.
	Gal(2.5)/Scop(0.63)	10	3.23	9	<0.05*
	Gal(10)/Scop(0.63)	7	3.73	6	<0.05*
(D) Galantamine/ITI	Solvent/15 min ITI	11	6.56	10	<0.001***
	Solvent/1 h ITI	9	1.24	8	n.s
	Gal(0.63)/15 min ITI	10	5.20	9	<0.01**
	Gal(0.63)/1 h ITI	8	5.97	7	<0.01**

Subsequent experiments: (A) retention interval (15 min, 1, 4 and 24 h); (B) scopolamine at various doses (0.04, 0.16 and 0.63 mg/kg); (C) combined galantamine (0.63, 2.5 and 10 mg/kg)/scopolamine (0.63 mg/kg) treatment; and (D) galantamine (0.63 mg/kg) with two different retention intervals (15 min and 1 h). ITI = intertrial or retention interval; scop = scopolamine; gal = galantamine. * is significantly different from zero at the 0.05 level; ** is significantly different from zero at the 0.01 level; *** is significantly different from zero at the 0.001 level; (*) is tendency to be different from zero $0.05 \le p \le 0.1$.

MANOVA General Linear Model (GLM) analysis (SPSS 11.5 Statistical Package) was used to determine the effects of the between subject factors (retention interval or intertrial interval [ITI] of 15 min, 1, 4 or 24 h; scopolamine/galantamine treatments), the within subject factors (test session: T1 and T2) and respective interactions on the dependent variables (d_2; total object exploration time on T1 and T2, e_1 and e_2, in s; the measure of locomotion, traveled distance during T1 and T2, in cm). One-way ANOVA was used to analyze group differences on separate time points. If appropriate, analyses were followed by post-hoc tests according to Dunnett to determine differences from respective control groups or according to Tukey to determine differences between experimental groups. Also, the number of animals with a discrimination index (d_2)>0.3 was determined within each experimental group. Finally, we tested with the One-Sample Test whether d_2 was equal to zero for each experimental group. Differences were considered as tendencies for $0.05 \leq p \leq 0.1$ and statistically significant for $p<0.05$.

3. Results

3.1. Experiment 1: Effects of different retention intervals on novel object exploration

3.1.1. Discrimination index (d_2)

The relative discrimination index (d_2) showed that there were no significant differences between groups in discrimination performance [$F(3,32)=2.43$, $p=0.084$]. However, the One-Sample Test (Table 1) indicated that d_2 was only significantly different from zero in the mice in the 15 min ITI condition ($p<0.001$), while d_2 was not significantly different from zero in the mice in the other ITI conditions. The number of mice with an index higher than 0.3 was: 8 (out of 10) in the 15 min ITI condition; 3 (out of 10) with the 1 h ITI; 2 (out of 8) with the 4 h ITI and 3 (out of 8) with the 24 h ITI (Fig. 1A).

3.1.2. Object exploration during T1 (e_1) and T2 (e_2)

Total time of exploration towards both objects did not change from T1 to T2 [$F(1,32)=0.003$, n.s.], and there were also no differences in habituation between ITI groups [$F(3,32)=1.05$, n.s.] in the mice (Fig. 2A).

3.1.3. Distance traveled

Overall, distance traveled decreased significantly from session T1 to T2 [$F(1,32)=16.08$, $p<0.001$] (Fig. 3A).

3.2. Experiment 2: Effects of scopolamine on novel object exploration

3.2.1. Discrimination index (d_2)

The relative discrimination index (d_2) showed that there was a significant difference between treatment groups in discrimination performance [$F(3,31)=5.31$, $p<0.01$]: d_2 tended to be higher in the solvent-treated mice in comparison with the animals treated with the lowest dose of scopolamine (0.04 mg/kg, $p=0.068$), while d_2 was significantly higher in the solvent-treated mice in comparison with the animals treated with the two highest doses of scopolamine (0.16 mg/kg, $p<0.05$; 0.63 mg/kg, $p<0.01$). The One-Sample Test (Table 1) indicated that d_2 was only significantly different from zero in the solvent-treated mice ($p<0.001$). The number of mice with an index higher than 0.3 was 6 (out of 8) with the solvent treatment; 4 (out of 8) with scopolamine (0.04 mg/kg); 3 (out of 10) with scopolamine (0.16 mg/kg) and zero (out of 10) with scopolamine (0.63 mg/kg) (Fig. 1B).

3.2.2. Object exploration during T1 (e_1) and T2 (e_2)

The total time of exploration towards both objects increased from T1 to T2 [$F(1,31)=5.03$, $p<0.05$]. The dose effect on the habituation parameter failed to reach significance [$F(3,31)=2.49$, $p=0.079$] (Fig. 2B).

3.2.3. Distance traveled

Overall, distance traveled decreased significantly from session T1 to T2 [$F(1,31)=6.33$, $p<0.05$]. However, on T1–T2, distance traveled was higher in mice treated with the two highest doses of scopolamine (0.16 and 0.63 mg/kg) in comparison with solvent-treated mice (all comparisons, $p<0.001$) (Fig. 3B).

3.3. Experiment 3: Reversal of scopolamine-induced novel object exploration deficits by galantamine

3.3.1. Discrimination index (d_2)

The relative discrimination index (d_2) showed that there was a significant difference between treatment groups in discrimination performance [$F(4,42)=5.70$, $p<0.001$]. The One-Sample Test (Table 1) indicated that d_2 was significantly different from zero in solvent-treated mice ($p<0.001$). Mice treated with scopolamine alone had a lower d_2 in comparison with the vehicle-treated animals ($p<0.001$), a value that was not different from zero. Also, d_2 was significantly lower in mice treated with galantamine (0.63 mg/kg)/scopolamine compared with the value in the vehicle-treated animals ($p<0.01$) (Fig. 1C).

In contrast, d_2 values in animals treated with galantamine (2.5 mg/kg)/scopolamine or mice treated with galantamine (10 mg/kg)/scopolamine did not differ significantly from the index in solvent-treated mice (2.5 mg/kg, $p=0.084$; 10 mg/kg, $p=0.631$), indicative of a normalization to control levels with these doses of galantamine. In addition, the One-Sample Test (Table 1) also indicated that d_2 was significantly different from zero in mice treated with galantamine (2.5 mg/kg)/scopolamine ($p<0.05$) and galantamine (10 mg/kg)/scopolamine ($p<0.05$). However, d_2 in galantamine (2.5 mg/kg)/scopolamine and galantamine (10 mg/kg)/scopolamine ($p=0.073$)-treated animals did not differ significantly with the value in mice treated with scopolamine alone. The number of mice with an index higher than 0.3 was 10 (out of 11) with the solvent treatment; 2 (out of 11) with scopolamine alone; 1 (out of 8) with galantamine (0.63 mg/kg)/scopolamine; 3 (out of 10) with galantamine (2.5 mg/kg)/scopolamine and 4 (out of 7) with galantamine (10 mg/kg)/scopolamine.

3.3.2. Object exploration during T1 (e_1) and T2 (e_2)

Total time of exploration towards both objects did not change from T1 to T2 [$F(1,42)=0.888$, n.s.], and there were

also no differences in habituation between experimental groups [$F(4,42)=1.46$, n.s.]. To check for effects of galantamine alone, some animals were also administered galantamine without scopolamine and tested in the setup (data not shown). It was observed that administration of the higher doses of galantamine (2.5 and 10 mg/kg) alone severely reduced exploration and locomotion in the mice (Fig. 2C).

3.3.3. Distance traveled

Overall, distance traveled did not change significantly from session T1 to T2 [$F(1,42)=0.056$, n.s.], while the interaction between treatment group and session did also not reach significance [$F(4,42)=1.63$, n.s.]. However, there were significant differences in distance between experimental groups [$F(4,42)=6.23$, $p<0.001$]. Distance traveled was significantly higher in the mice treated with galantamine (0.63 mg/kg)/scopolamine than in solvent-treated mice ($p<0.01$) (Fig. 3C).

3.4. Experiment 4: Effects of galantamine on novel object exploration with the retention intervals of 15 min and 1 h

3.4.1. Discrimination index (d_2)

The relative discrimination index (d_2) showed that there were no significant main effects of galantamine [$F(1,34)=1.69$, n.s.] or ITI [$F(1,34)=2.06$, n.s.]. Also, the interaction between both factors failed to reach significance [$F(1,34)=2.31$, n.s]. The One-Sample Test (Table 1), however, indicated that d_2 was significantly different from zero in the solvent/15 min ITI group ($p<0.001$), galantamine (0.63 mg/kg)/15 min ITI group ($p<0.01$) and galantamine (0.63 mg/kg)/1 h ITI group ($p<0.01$), but not in the solvent/1 h ITI condition. The number of mice with an index higher than 0.3 was: 10 (out of 11) in the solvent/15 min ITI condition; 4 (out of 9) in the solvent/1 h ITI group; 8 (out of 10) in the galantamine (0.63 mg/kg)/15 min ITI group and 6 (out of 8) in the galantamine (0.63 mg/kg)/1 h ITI group (Fig. 1D).

3.4.2. Object exploration during T1 (e_1) and T2 (e_2)

Total time of exploration towards both objects did not change from T1 to T2 [$F(1,34)=0.776$, n.s.], and there was also no significant difference in habituation between experimental groups [$F(1,34)=2.93$, $p=0.096$]. Neither of the other effects (and interactions) reached significance for total exploration time (Fig. 2D).

3.4.3. Distance traveled

Overall, distance traveled decreased significantly from session T1 to T2 [$F(1,34)=43.63$, $p<0.001$]. Only in the 1 h ITI condition, distance traveled decreased significantly from

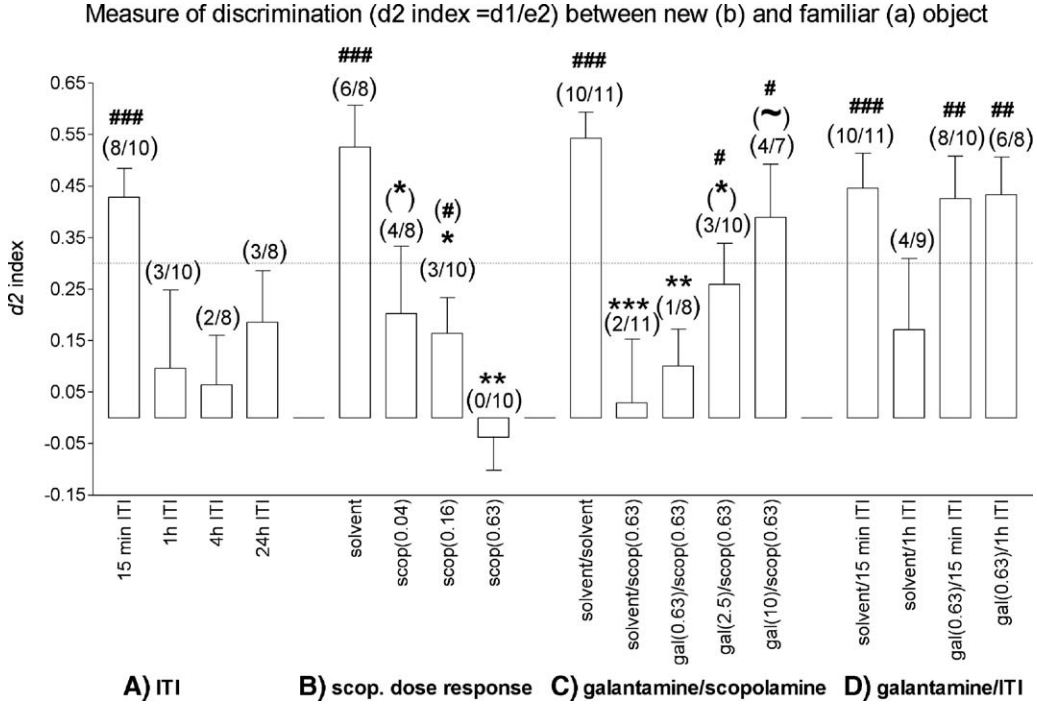

Fig. 1. A–D: The discrimination index d_2 between the new and familiar object in subsequent experiments: effects of the (A) retention interval (15 min, 1, 4 and 24 h), (B) scopolamine at various doses (0.04, 0.16 and 0.63 mg/kg), (C) combined galantamine (0.63, 2.5 and 10 mg/kg)/scopolamine (0.63 mg/kg) treatment and (D) galantamine (0.63 mg/kg) with two different retention intervals (15 min and 1 h). Drugs were administered subcutaneously, 30 min prior to the acquisition session (doses are indicated between brackets, in mg/kg). Also indicated between brackets: number of mice with an index higher than 0.3 (from the total group of mice in each experimental group). $d_2=d_1/e_2$, where d_1 is the measure of discrimination between the new (b) and familiar (a) object ($d_1=$b-a); e_2 is the time spend in exploring both the familiar and new object in the second trial ($e_2=$a+b); ITI=intertrial or retention interval; scop=scopolamine; gal=galantamine. * is significantly different from the solvent group at the 0.05 level; ** is significantly different from the solvent group at the 0.01 level; *** is significantly different from the solvent group at the 0.001 level; (*) is tendency to be different from the solvent group $0.05\leq p\leq 0.1$; (~) is tendency to be different from the scopolamine-treated mice $0.05\leq p\leq 0.1$; # is significantly different from zero at the 0.05 level; ## is significantly different from zero at the 0.01 level; ### is significantly different from zero at the 0.001 level; (#) is tendency to be different from zero $0.05\leq p\leq 0.1$.

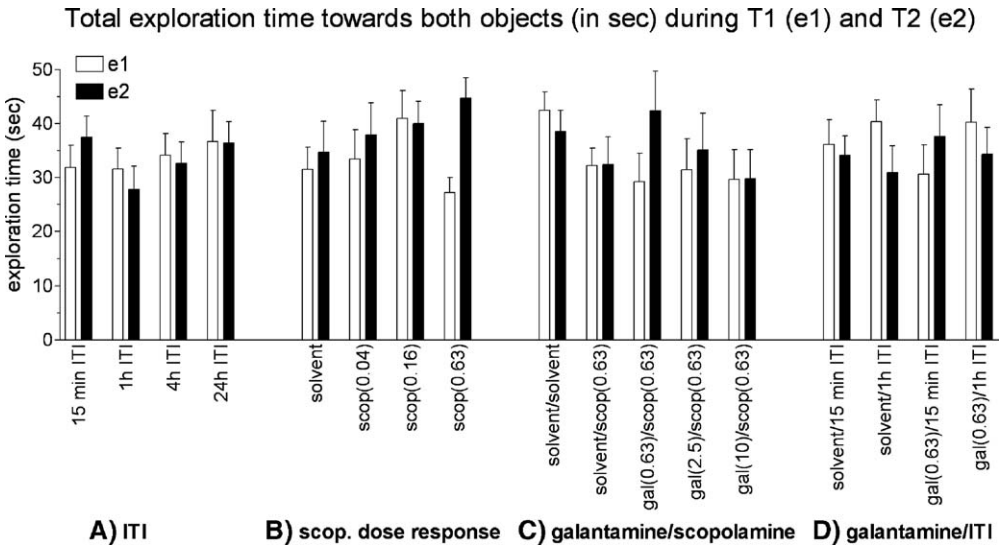

Fig. 2. A–D: Total exploration time towards both objects (in s) during T1 (e_1) and T2 (e_2): effects of the (A) retention interval (15 min, 1, 4 and 24 h), (B) scopolamine at various doses (0.04, 0.16 and 0.63 mg/kg), (C) combined galantamine (0.63, 2.5 and 10 mg/kg)/scopolamine (0.63 mg/kg) treatment and (D) galantamine (0.63 mg/kg) with two different retention intervals (15 min and 1 h). Drugs were administered subcutaneously, 30 min prior to the acquisition session (doses are indicated between brackets, in mg/kg). e_1 is the time spend in exploring both identical objects ($e_1 = a_1 + a_2$) in the first trial (T1); e_2 is the time spend in exploring both the familiar (a) and new (b) object ($e_2 = a + b$) in the second trial (T2); ITI = intertrial or retention interval; scop = scopolamine; gal = galantamine.

session T1 to T2 (ITI by session interaction [$F(1,34) = 20.29$, $p < 0.001$]) (Fig. 3D).

4. Discussion

4.1. Prolonging the retention interval

The first aim in the present study was to establish the retention interval (ITI, 15 min, 1 h, 4 h or 24 h) at which optimal versus reduced performance occurred. Discrimination between the novel and the familiar object was very clear in Swiss mice when using the 15 min ITI, but absent at the 1 h ITI, indicating that retention was reduced with this longer interval. In contrast, Sik et al. (2003) have tested the memory performance of different mouse strains (129/Sv, BALB/c, C57BL and Swiss) and have found that mice still showed a good object memory performance when a 1 h retention interval was interposed between the two trials, while when a 24 h retention interval was used, mice did not discriminate between the novel and the familiar object in the second trial. Absence of retention of the sample object at the 1 h ITI in the current study can potentially be explained by the fact that the objects were not dissimilar

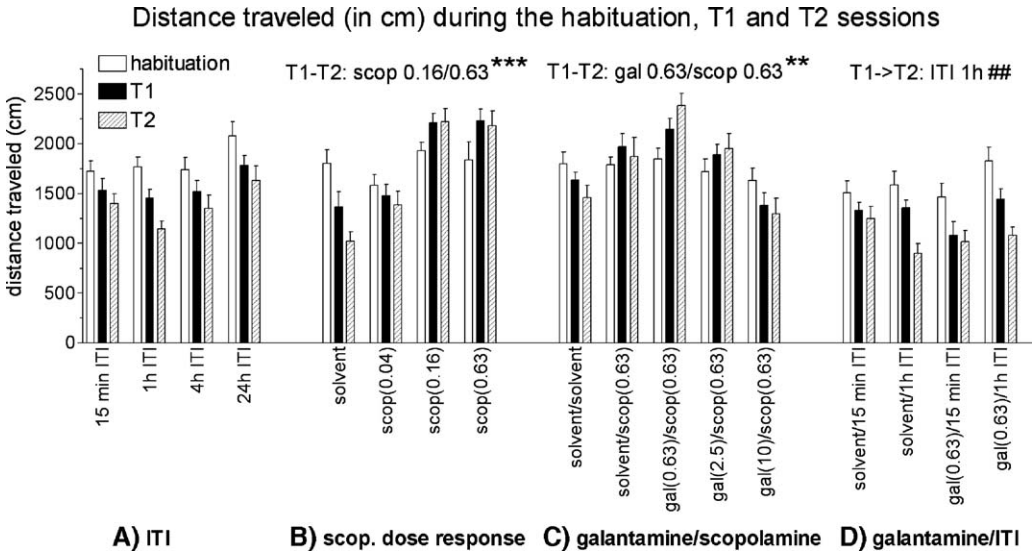

Fig. 3. A–D: Distance traveled (in cm) during the habituation, T1 and T2 sessions: effects of the (A) retention interval (15 min, 1, 4 and 24 h), (B) scopolamine at various doses (0.04, 0.16 and 0.63 mg/kg), (C) combined galantamine (0.63, 2.5 and 10 mg/kg)/scopolamine (0.63 mg/kg) treatment and (D) galantamine (0.63 mg/kg) with two different retention intervals (15 min and 1 h). Drugs were administered subcutaneously, 30 min. prior to the acquisition session (doses are indicated between brackets, in mg/kg). T1 = first trial; T2 = second trial; ITI = intertrial or retention interval; scop = scopolamine; gal = galantamine. ** is significantly different from the solvent group at the 0.01 level; *** is significantly different from the solvent group at the 0.001 level; ## is significantly different between T1 and T2 at the 0.01 level.

enough. Thus, this could have resulted in a reduced discrimination between the novel and familiar object at a shorter ITI of 1 h. This hypothesis can be further tested in future studies by using more distinct objects than the ones used here.

4.2. Scopolamine disruption

In the following experiment, the dose of scopolamine was determined at which deficits were found in object recognition memory using the short 15 min ITI in Swiss mice. Deficits in object discrimination were most prominent with the highest dose of scopolamine (0.63 mg/kg, s.c., administered 30 min prior to trial 1). Also, the two higher doses of scopolamine both increased distance traveled during T1 and T2 in comparison with solvent-treated mice. Similar results have been reported in Swiss mice by others as well (Dodart et al., 1997): administration of scopolamine (0.3, 1 and 3 mg/kg, s.c.) before trial 1 reduced recognition performance on trial 2 and induced other behavioral effects, including an increase in locomotor activity. Rats have also been shown to be unable to discriminate between familiar and novel objects following scopolamine (0.1-0.2 mg/kg, s.c./i.p.) administration (Bartolini et al., 1996; Lieben, et al., 2005; Rispoli et al., 2004). A potential location of action of scopolamine has been proposed, based on a study in which the drug was injected into the perirhinal cortex of rats, which significantly impaired discrimination in the ORT (Abe et al., 2004). Also, in humans, the suggestion has been put forward that the perirhinal cortex plays a critical role in object recognition memory (Buffalo et al., 1998). The present results confirm that the ORT is a useful model to test recognition memory in mice and that blocking the central cholinergic system by scopolamine impairs this form of memory (Dodart et al., 1997).

4.3. Disruption models: retention interval versus scopolamine

The choice for a particular disruption model (either retention interval or scopolamine or another one) is dependent on (the stage of) the disease of interest. McDonald and Overmier (1998) have stated that “although scopolamine administration may be an attractive model, both because of the global impairment and neurochemical correspondence with AD, it may not be ideal to model the mnemonic deficits in the early stages of AD.” In the review of McDonald and Overmier (1998), the authors conclude that “early-stage AD patients, in particular, show increased impairment relative to controls as the delay increases”. This would suggest that the retention interval model in the ORT, would be a better model for early-stage AD. It can be speculated that compounds that ameliorate the spontaneous retention deficits will be better treatments for early AD than drugs that solely reduce scopolamine-induced disturbances.

In the case of schizophrenia, the use of other models might be more relevant: for instance, disturbances in object recognition have been found with lesions of the ventral hippocampus in neonatal rat pups (nVH) that result in post-pubertal alterations in a variety of behaviors that bear some analogy to schizophrenia (Bhardwaj et al., 2005). The same holds true for subchronic treatment with phencyclidine (PCP, 2 mg/kg, twice daily for a week)(Idris, et al., 2005). So, evidence for efficacy of a certain drug in multiple disruption models would suggest that the drug might be active in a broader variety of patients. In addition, the mechanism of action of the drug would provide information on the underlying neurobiological substrate of the mnemonic deficit in particular patient groups when tested in multiple disease models.

4.4. Galantamine as a positive control

Potential reversal with different doses of galantamine (0.63, 2.5 and 10 mg/kg, s.c., T-30) of scopolamine -induced deficits was investigated. We have found that particularly the highest dose of galantamine (10 mg/kg) normalized retention performance to control levels. Although the discrimination index in scopolamine-galantamine 10 mg/kg-treated animals did not differ significantly with the value in mice treated with scopolamine alone, there was a tendency for a difference, which indicates that there was a partial reversal of the effects of scopolamine on the discrimination index. Increasing the number of animals per group would have probably resulted in more prominent effects. Administration of the higher doses of galantamine (2.5 and 10 mg/kg) alone severely reduced exploration and locomotion in the mice (data not shown). This is not surprising, but rather in accordance with inverted U-shaped dose-response curves described for cholinomimetics (Van Dam et al., 2005). Galantamine has also been found to exert similar effects in other cognitive paradigms. For example, galantamine-treated normal animals exhibited significantly impaired performance as compared to untreated controls in a shuttle-box test (Iliev et al., 1999). Also, in contrast to the beneficial effects of galantamine in nucleus Basalis of Meynert (nBM)-lesioned mice, galantamine (5.0 mg/kg, i.p.) impaired performance of control mice in the reversal phase of the modified Morris swim task, as motor activity was severely reduced (Sweeney et al., 1989).

Finally, galantamine (0.63 mg/kg), at a dose that was not expected to induce side effects (reduced object exploration and locomotion), was also investigated when two different retention intervals were used: the ITI of 15 min with very good performance and the 1 h ITI at which sub-optimal object recognition memory occurred. Galantamine (0.63 mg/kg) indeed had no adverse effects as tested in the shorter 15 min ITI condition. In the 1 h ITI condition, solvent-treated mice did not significantly discriminate between the novel and the familiar object (discrimination index was equal to zero), while galantamine (0.63 mg/kg)-treated mice attained a discrimination index which was significantly higher than zero, exhibiting a good object recognition memory.

In sum, galantamine was shown to possess episodic memory enhancing effects in two conditions that reduced object discrimination: galantamine 10 mg/kg normalized the discrimination index to control levels when scopolamine was co-administered and the index reached a significantly higher value than zero with galantamine 0.63 mg/kg when the longer retention interval of 1 h was used. Similarly, (−)-9-dehydrogalanthaminium bromide, a synthetic galantamine derivative, was reported to improve object recognition in rats, via an enhancement of acquisition (Lamirault et al., 2003). Recently, Selcher et al. (2005) have also reported that

the AChEIs donepezil, rivastigmine and galantamine were all efficacious in improving retention in the ORT in rats.

In conclusion, galantamine can be used as a positive control in the ORT. Subsequently, potential novel cognitive enhancing drugs can be tested for their efficacy in both disruption models.

4.5. Conclusion

Use of the ORT as a pre-clinical cognitive test can be an interesting approach to evaluate potential novel cognitive-enhancing compounds. In this study, galantamine was shown to possess beneficial effects in two conditions that reduced object recognition memory: scopolamine-induced deficits and when a longer retention interval was used. Therefore, galantamine can potentially be considered as a positive control in the ORT.

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