



Room novelty, sex, scopolamine and their interactions as determinants of general activity and rearing, and light–dark preferences in rats

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Abstract

Male and female rats were assessed for effects of scopolamine on general activity, rearing and light–dark preferences when tested in either a familiar or a novel room. Males but not females reared more often when tested in the familiar rather than novel room, and the response was increased by scopolamine for all rats combined. Whereas scopolamine increased general activity for females (but not males) in the familiar room, it decreased the response for males (but not females) in the novel room. Females crossed more often between the dark and light sides of a light–dark box and, when treated with saline but not drug, spent more time in the light side than males. Scopolamine reduced the amount of time spent in the light side for females only. While the results were discussed mainly in terms of sex differences in fearfulness, their principal value was in demonstrating the effectiveness of room novelty and sex in determining levels of the behaviors recorded, and drug responsiveness.

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1. Introduction

In spite of abundant long-standing evidence that the extent and direction of drug effects on the behavior of laboratory animals can be influenced by a range of non-pharmacological influences such as rearing conditions (Baumel et al., 1969; Hughes and Syme, 1972; Lowe and Williams, 1972), housing (Faraday et al., 1999) and testing environment (Brown, 1960; Sahakian and Robbins, 1975; Syme and Syme, 1973), there still appears to be relatively little awareness of this. Non-pharmacological influences are particularly important in studies of unconditioned behavior that do

not involve animals being required to learn responses. This is because the relative lack of constraints that are inevitably present when animals are trained to respond are relatively lacking thereby facilitating the occurrence (or not) of more freely chosen ways of behaving. Consequently, such unconditioned behavior is particularly susceptible to the effects of a range of extraneous influences. For example, measures of non-specific general activity, locomotion and stimulus preferences are especially prone to modification by a variety of procedural and subject variables, such as novelty of an animal's testing environment. It was recently shown that levels of locomotor activity in male rats were dependent on the novelty of the room and the cages in which the animals were tested (Galani et al., 2001). The authors therefore recommended that such influences should be taken into account when assess-

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ing the effects of drugs. Their advice is supported by a much earlier report of the influence of room and experimenter novelty in determining effects of methamphetamine on general activity in female rats (Hughes and Beveridge, 1980). This study showed that, when tested in a familiar room, the drug increased activity irrespective of the novelty of the experimenter. However, when tested in a novel room, the drug increased activity only in those rats tested by a familiar experimenter.

A very significant but frequently ignored non-pharmacological influence on drug action is the sex of experimental animals (Broadhurst, 1978; Claassen, 1994; Wilson et al., 1999). There are many cases on record where effects of various drugs are different in the two sexes. For example, while 5 mg/kg i.p. chlor-diazepoxide was shown to decrease occupancy of the novel half of an exploration box in male hooded rats, females were unaffected by the drug (Hughes and Syme, 1972). More recently, responsiveness to a Y-maze arm that had changed in brightness from a previous acquisition trial was enhanced in male rats by pre- and post-acquisition treatment with 5 but not 10 mg/kg i.p. D-cycloserine, whereas the reverse dose-related outcome characterized females (Hughes, *in press*). Nevertheless, most investigations still involve male subjects exclusively.

Because scopolamine is an antimuscarinic drug that interferes with hippocampal cholinergic processes involved in memory (Hagan and Morris, 1988) it is widely used in memory experimentation, as well as in other areas of interest (such as attention, Cheal (1981), or inhibition, Milar et al. (1978)). But even though females may be more sensitive than males to scopolamine (Berger-Sweeney et al., 1995), neither sex nor room novelty differences have been seriously considered as possible determinants of the drug's effects on most forms of unconditioned behavior. The present study was therefore designed to assess their importance with respect to measures of general activity, rearing and light–dark preference.

Although estimates of non-specific general activity are useful in assessing whether or not drugs may have central effects (Maxwell, 1968) worthy of further investigation, there can be considerable variation in their effectiveness. It is possible that such variations may be due in part to the influence of non-pharmacological variables, such as those noted above. The frequency of

rearing is also a common measure of drug effects on vertical activity which can depend to some extent on the operation of environmental or genetic influences (Hughes, 1982).

Measures of tendencies for rodents to leave black or darkened environments to enter white or illuminated spaces exploit a conflict between their natural curiosity about a novel environment, and their fear of bright light (Hascoët et al., 2001; Sanchez, 1996). Consequently, tests involving such measures have been extensively used in the evaluation of anxiolytic and anxiogenic drugs (Flausino et al., 2002; Hascoët and Bourin, 1998). However, there is inconsistency amongst many of the outcomes, possibly because of the influence of non-pharmacological variables (Hascoët and Bourin, 1998).

Scopolamine has been shown to increase general activity (Hughes, 1982) and reduce rearing up vertically on the hind legs in rats (Horsburgh and Hughes, 1981; Hughes et al., 1975). Although it is usually assumed that behavioral changes following systemic administration of scopolamine arise from its action on specific brain neurochemical systems, there is evidence that rats may find its action aversive (Berger, 1972; MacMahon et al., 1981). Such effects can produce novelty avoidance in the absence of memory deficits that appears to be mainly due to the drug's peripheral rather than central action (Horsburgh and Hughes, 1981; Hughes et al., 1989; MacMahon et al., 1981). Consequently, it is likely to be anxiogenic and thus of possible interest in terms of its effects on light–dark preferences.

2. Materials and methods

2.1. Subjects

The subjects were 14 male and 14 female Wistar albino rats bred in the Psychology Department of the University of Canterbury, and approximately 120 days old at the beginning of testing. At the beginning of testing, the mean (\pm S.E.) bodyweights for males and females were 337.18 (\pm 5.60) g and 259.67 (\pm 5.32) g, respectively. They were caged in groups of two or three same-sexed animals with free access to food and water in 12 h light/12 h dark conditions, and an ambient temperature of 20 ± 1 °C. Apart from that necessitated

by routine cage-cleaning and the identification of individuals (by means of sheep branding dyes applied to their fur), the rats were not regularly handled prior to the experiment. All testing occurred during the dark phase of their light/dark cycle.

2.2. Apparatus

2.2.1. General

The rats were tested in either a general activity chamber or a light–dark box. Each piece of apparatus sat on a trolley that could be wheeled between the two rooms where all testing took place. Even illumination was provided by normal room fluorescent lighting approximately 1.5 m overhead.

2.2.2. Activity chamber

General activity and rearing were measured in a 200 mm × 200 mm clear Perspex 380-mm high box that was open at both the top and the bottom. The floor consisted of a removable sheet of absorbent white paper. To enable the measurement of rearing behavior, two horizontal black lines had been drawn around the walls at heights of 110 mm (for females) and 140 mm (for males) from the floor. These heights were based on earlier observations of the distance which, because of differences in the rats' size and thus length of their forelegs, members of each sex needed to move vertically so that both front paws lost contact with the floor. At the top of the chamber, in opposing corners, were attached an ultrasonic transmitter and receiver operating at a nominal frequency of 40 kHz (CFP Activity Monitor, model 418/8181). Disturbances of the ultrasonic sound field by a rat's movement within the chamber were recorded by a Lafayette counter (model 54417) connected to the receiver. According to the manufacture's specifications, there is no evidence that the ultrasonic field itself affects rats' behavior.

2.2.3. Light–dark box

Light–dark preferences were recorded in a clear varnished wooden box comprising two 300-mm long × 200-mm wide × 300-mm high compartments. The compartments were separated by a wooden partition in which there was a 100 mm × 100 mm doorway that could be closed by means of a guillotine slide. One compartment was covered by a hinged wooden lid (the dark side), and the other was covered by a hinged clear Perspex lid (the light side).

2.3. Procedure

2.3.1. General

Half the rats of each sex were randomly chosen for testing in the activity chamber, and the other half for testing in the light–dark box. Following a 2 ml/kg i.p. injection of isotonic saline, or either 1.0 or 2.0 mg/kg scopolamine hydrobromide (in saline), each rat was returned to its home-cage. Fifteen min later, it was taken to the appropriate apparatus that was set up in either the room where the rat was normally housed (the familiar room), or in a nearby spatially identical room that was unfamiliar because of the lack of olfactory, visual and auditory stimuli arising from presence in the familiar room of other rats and cages (the novel room). Prior to all testing in the familiar room, the observer took an extra 14–16 paces, while holding the rat, before placing it in the apparatus. This was to take account of the extra distance traveled between the rat's cage and the apparatus when it was transported to the novel room.

All rats experienced one 5-min trial following administration of each of the three doses of scopolamine in each of the testing rooms (i.e., a total of 6 trials), with an interval of one week between each of their trials. On any one testing day they were injected with the same dose in squads of 3, with 6 min between each injection.

2.3.2. Rearing and general activity

The rat was placed into the center of the activity chamber with the observer seated 1.5 m away at the same eye-level as the lines on the walls. After 10 s, the activity monitor was switched on and the number of times the rat's head appeared above the line appropriate for its sex was counted. This measure ensured that each one of its front paws had lost contact with the floor thereby satisfying the criterion for "rearing," namely vertical movement with neither front paw on the floor. At the end of the 5-min trial, the rat was returned to its home cage, a clean paper floor was provided, and the inside walls of the apparatus were washed and dried.

2.3.3. Light–dark preferences

The rat was placed into the dark side of the light–dark box and, 10 s later, the guillotine slide was removed. The observer stood 1.5 m from the appara-

tus facing both sides and, by means of a hand-held counter and timer, recorded the latency of emergence from the dark side, the number of transitions between the two sides, and the total time spent in the light side. At the end of the 5-min trial, the rat was returned to its home cage, and both sides of the box were washed and dried.

3. Results

Preliminary one-way repeated measures ANOVAs applied separately to each measure failed to reveal any evidence of order effects over the 6 weekly trials independent of the randomly experienced experimental conditions. Therefore, all measures were subjected to separate sex \times room \times dose ANOVAs for repeated measures.

3.1. Rearing and general activity

Main effects of sex, testing room and scopolamine on rearing and general activity, and results of the analyses can be seen in Table 1.

Females reared significantly more often than males, and all rats reared more often when tested in the familiar than in the novel room. However, these differences are more appropriately considered in the light of a sig-

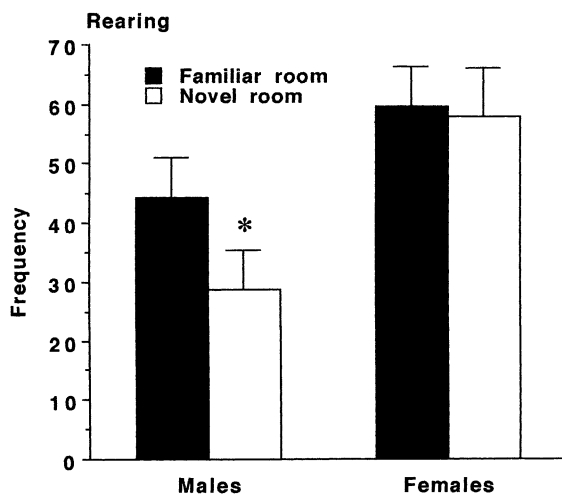


Fig. 1. Mean (\pm S.E.) frequencies of rearing in the familiar and novel rooms in males and females separately. *Significantly different (Scheffé test, $P < 0.05$) from the familiar room frequency.

nificant sex \times room type interaction ($F(1, 12) = 7.41$, $P < 0.02$) outlined in Fig. 1.

Post hoc Scheffé tests ($P < 0.05$) showed that, while males reared significantly more often when tested in the familiar than in the novel room, this difference did not characterize females. Rearing was significantly increased by both doses of scopolamine for all rats combined ($P < 0.05$, Scheffé tests).

Table 1

Mean (\pm S.E.) frequencies of rearing and levels of general activity for each sex, type of room and dose of scopolamine, and results of F -tests

	Sex		$F(1,12)$	P
	Males	Females		
Rearing ^a	36.4 (6.5)	58.9 (7.1)	5.46	0.038
General activity ^b	355.2 (14.5)	372.1 (10.2)	0.91	0.361
	Room type			
	Familiar	Novel		
Rearing ^a	51.98 (5.0)	43.3 (6.5)	12.20	0.004
General activity ^b	369.9 (10.3)	357.4 (18.9)	0.38	0.547
	Scopolamine dose			$F(2,24)$
	0 mg/kg	1.0 mg/kg	2.0 mg/kg	
Rearing	24.1 (2.5)	58.5 (8.2)	60.3 (7.7)	22.71
General activity ^b	292.2 (19.0)	383.0 (11.0)	415.8 (11.5)	22.24

^a Sex \times room interaction significant.

^b Sex \times room \times dose interaction significant.

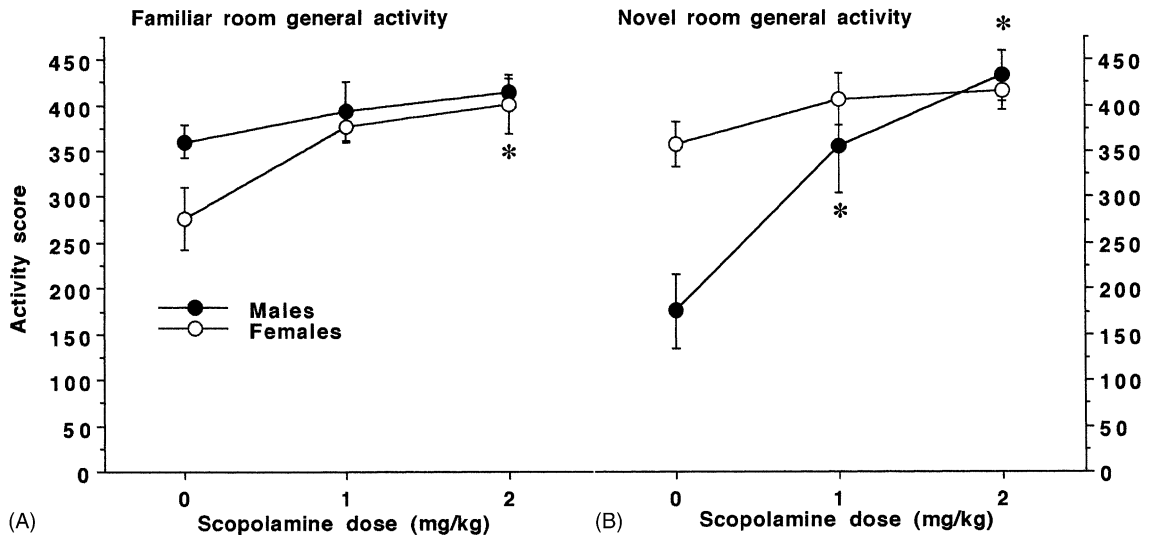


Fig. 2. Mean (\pm S.E.) levels of general activity for males and females separately in (A) the familiar room and (B) the novel room following treatment with scopolamine. *Significantly different (Scheffé tests, $P < 0.05$) from the 0 mg/kg control condition.

Neither the sex nor room-type main effect was significant for general activity which, was nevertheless increased by both doses of scopolamine. However, this latter effect should be interpreted in terms of a significant three-way interaction between all independent variables ($F(2, 24) = 5.13$, $P < 0.015$, see Fig. 2).

In view of the differences outlined in Fig. 2 between males and females in the way scopolamine affected their activity in the familiar and in the novel room, post hoc one-way ANOVAs were performed to assess the significance of the apparent sex-dependent drug effect in each of the two rooms separately. These revealed a significant effect for females when tested in the familiar room ($F(2, 12) = 5.69$, $P < 0.02$) due to an increase following treatment with 2.0 mg/kg, but not in the novel room ($F(2, 12) = 1.35$, $P > 0.25$). However, the reverse situation prevailed for males namely, a significant effect when tested in the novel room ($F(2, 12) = 15.64$, $P < 0.001$) due to an increase with both doses, but no effect in the familiar room ($F(2, 12) = 0.97$, $P > 0.5$).

3.2. Light–dark preferences

Main effects of sex, testing room and scopolamine on latency of first entering the light side, transitions between the two sides and total time spent in the

light side, and results of the ANOVAs can be seen in Table 2.

While latency of first entering the light side was not affected by any of the independent variables, transitions were higher for females than for males, and lower for all rats combined following treatment with 2.0 mg/kg scopolamine ($P < 0.05$, Scheffé test). Total time spent in the light side was higher in females than in males, and lower with both doses of scopolamine (<0.05 , Scheffé tests). But a significant sex \times scopolamine interaction ($F(2, 24) = 3.47$, $P < 0.05$, see Fig. 3) followed by one-way ANOVAs for each sex separately revealed that both doses of scopolamine significantly reduced time in the light for female but not for male rats.

4. Discussion

It is clear that both the sex of the rats and their treatment with scopolamine affected their behavior in both types of apparatus. Additionally, responses emitted in the activity chamber were influenced by the nature of the room in which the rats were tested. The higher frequency of rearing in this chamber (which of course also contributed to the general activity score), and the greater number of transitions between the two sides

Table 2

Mean (\pm S.E.) emergence latencies, light–dark transitions and time spent in the light for each sex, type of room and dose of scopolamine, and results of *F*-tests

	Sex		<i>F</i> (1,12)	<i>P</i>
	Males	Females		
Latency	46.3 (14.5)	21.25 (6.8)	2.44	0.144
Transitions	0.8 (0.3)	2.0 (0.3)	7.41	0.019
Time in light ^a	9.8 (3.6)	25.3 (5.5)	5.66	0.035
Room type				
	Familiar	Novel		
Latency	36.8 (12.0)	30.79 (8.0)	0.29	0.603
Transitions	1.4 (0.3)	1.4 (0.3)	0.01	0.941
Time in light	17.3 (3.7)	17.8 (4.7)	0.02	0.878
Scopolamine dose				
	0 mg/kg	1.0 mg/kg	2.0 mg/kg	
Latency	37.4 (10.8)	28.2 (11.6)	46.4 (23.9)	0.22
Transitions	2.5 (0.6)	1.3 (0.4)	0.9 (0.2)	5.34
Time in light ^a	38.3 (10.9)	9.0 (2.7)	5.4 (1.9)	9.14

^a Sex \times drug interaction significant.

in the light–dark box shown by female rats were consistent with sex differences in activity reported previously (Archer, 1975). Although higher rearing levels in female compared with male rats is usually attributed

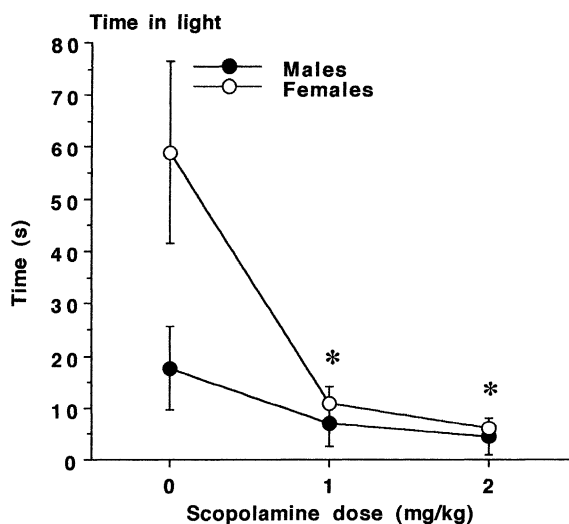


Fig. 3. Mean (\pm S.E.) time spent in the light side of the light–dark box for males and females separately following treatment with scopolamine. *Significantly different (Scheffé tests, $P < 0.05$) from the 0 mg/kg control condition.

to the former sex's lower fear or stronger exploratory tendencies (Archer, 1975), it is possible that, because of their lighter body weights, females had been more inclined to move in a vertical direction. However, re-examination of data from an earlier unpublished study failed to support this suggestion. The frequency of rearing (assessed in exactly the same way as in the present study) was correlated with body weight for 11 male (mean (\pm S.E.) weight = 322.64 (\pm 6.76) g) and 12 female (mean (\pm S.E.) weight = 256.17 (\pm 5.60) g) Wistar albino rats. While the sex difference in rearing was significant (males = 19.00 (\pm 2.44), females = 26.25 (\pm 2.47), $t(21) = 2.08$, $P < 0.05$), the correlation did not achieve significance for either males ($r = 0.04$, d.f. = 10) or females ($r = -0.06$, d.f. = 11). A similar lack of a relationship between body weight and number of vertical movements was reported for male rats by Levin (1991).

Only male rearing in the activity chamber was reduced when the rats were tested in the novel room, thereby suggesting a sex difference in responsiveness to cues outside of the apparatus, such as the lack of other rats and cages. It is possible that this difference was due to a neophobic suppression of the response in males alone because of their greater fearfulness, suggested by some authors (Aguilar et al., 2003; Gray,

1971; Russell, 1975). Alternatively, females may have habituated more rapidly to the novel room stimuli (Hughes, 1999), thus diminishing the suppressing effects of any initial neophobic reaction they might have had.

The overall scopolamine-induced increase in rearing was contrary to several earlier observations in different experimental settings (Bryan and Ellison, 1975; Hughes et al., 1975; Stewart, 1977), but consistent with others (Stewart, 1975; van Abeelen and Strijbosch, 1969), thereby supporting claims of dependence of the drug's effects on the apparatus used (Hughes, 1982).

The dependence of scopolamine on sex and room type in determining its effects on general activity is a strong indication of the extent to which non-pharmacological factors can influence a drug's action. In most behavioral pharmacological investigations, mainly male rodents are tested in novel rooms following drug administration. In the present study, when scopolamine-injected rats were tested in the novel room, the results for males were consistent with many previous studies, namely an increase in general activity (Hughes, 1982). However, the drug had no effect on females. But when the rats were tested in a familiar room, scopolamine only increased general activity in females. The importance of sex and room novelty in determining scopolamine's effects on general activity might help account for some of the earlier reported inconsistencies in this respect (for a review see Hughes, 1982). From inspection of Fig. 2, it would appear that, the drug's action for the two sexes when tested in each room was in accord with the law of initial value (Wilder, 1957). In other words, a low initial (or untreated) value for females alone in the familiar room, and a low initial value for males alone in the novel room, and thus increases with scopolamine in both cases. Movement from the familiar to the novel room resulted in decreased and increased activity for males and females, respectively when confronted with the novel room cues. This sex difference may have arisen from a neophobic response in the possibly more fearful males (Russell, 1975) when tested in the novel room which led to suppression of behavior in the saline condition sufficient to enable activity enhancement by scopolamine. However, in the familiar and possibly less fear-inducing room, the males' relatively high saline level of activ-

ity left little opportunity for further enhancement by scopolamine. On the other hand, due to more rapid habituation to the familiar room cues, females may have been less active when treated with saline and thus more responsive to scopolamine. Then when tested after saline injection in the novel room, their possibly lower level of fearfulness (compared with males) may have enabled stimulation rather than suppression of activity to a level that prevented further increases with scopolamine.

The greater number of transitions between the two sides of the light–dark box and, for saline-treated animals only, the longer time spent in the light side by females are consistent with their higher activity (Archer, 1975) and lower fearfulness (Aguilar et al., 2003; Gray, 1971; Russell, 1975), respectively, compared with males. While the decrease in transitions with scopolamine is contrary to its effects on general or locomotor activity, as shown in the present study and many previous reports (for a review see Hughes, 1982), the result suggests avoidance of the light side thereby supporting a possible aversive or anxiogenic action of the drug (Berger, 1972; MacMahon et al., 1981). However, the sex-related decline (favoring females) in time spent in the light side suggests that any aversive action was specific to females. But from inspection of Fig. 3, it is clear that the sex difference in reactivity to scopolamine arose from the low initial value of the response for males who appeared to find the light side no less aversive with saline than with the drug. Consequently, any further scopolamine-induced reduction in the time spent in the light side was only possible for females. It is possible that any aversiveness of scopolamine's action and hence avoidance of the light side of the box was caused by hypersensitivity to light arising from the drug's mydriatic effects (Horsburgh and Hughes, 1980; Oliverio, 1968). This effect may have also played a part in the drug's suppression of rearing in the activity chamber, as the response would have inevitably brought each rat's eyes closer to the overhead room lighting.

Irrespective of the particular reasons for the results obtained in the present study, it is clear that rearing, general activity and light–dark preferences in rats can depend on their sex and the nature of the room in which they are tested. The influence of room novelty supports earlier findings (Galani et al., 2001) as well as demonstrating that it can also determine

some effects of scopolamine, as has been shown for methamphetamine (Hughes and Beveridge, 1980). The sex-dependent effects of scopolamine are in line with some earlier findings (Berger-Sweeney et al., 1995) and may support evidence of estrogen involvement in the drug's action for females (Fader et al., 1999; Voytko, 2002). However, in the present study, the outcomes are more likely to have arisen from the operation of the Law of Initial Values (Wilder, 1957), although it is possible that the initial values for sex may have been related to hormonal differences. The results for scopolamine further extend the range of pharmacological agents and behavioral situations for which interactions between sex and drug action have been described (e.g., Arenas et al., 1993; Booze et al., 1999; Hughes, in press; Schindler and Carmona, 2002). In future research it is therefore advisable to take account of sex and room novelty when assessing the effects of scopolamine and other drugs on the specific responses recorded and, more generally, on other forms of behavior that do not rely on learning. Also, as suggested by the time spent in the light side of the light–dark box, rats' sex could play a part in effects of scopolamine on preference tasks that might be potentially influenced by any fear-related avoidance of bright light possibly arising from the drug's aversive action (Berger, 1972; MacMahon et al., 1981).

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