

Research report

Responsiveness to brightness change in male and female rats following treatment with the partial agonist of the *N*-methyl-D-aspartate receptor, D-cycloserine

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Abstract

Male and female hooded rats were intraperitoneally injected with 0 (vehicle only), 5 or 10 mg/kg D-cycloserine (DCS) and then individually allowed free access to the arms of a Y maze (acquisition trial), one of which was black and the other white. Their ability to later recognize the arm that had changed from white to black was assessed from the first arm entered, and the number of times the novel changed arm was repeatedly entered as well as the total time they spent in this arm. DCS increased the number of times the novel arm was entered first and, at the higher dose, repeated entries of and time spent in this arm by female rats. Males showed increases after the lower but not higher dose. In a second experiment, DCS was administered after rather than before the acquisition trial. With the exception of the first arm entered for males only, DCS did not significantly affect choices of the novel arm. However, contrary to treatment with vehicle, such choices were significantly higher than chance expectancies after 5 mg/kg DCS, thereby indicating that the treated rats were able to recognize the novel arm. It was concluded that, in the first experiment, DCS had mainly improved attention and/or encoding, and had slightly enhanced memory in the second. Any effects on memory were most likely due to prevention of forgetting. There was also evidence of anxiolytic effects of DCS that may have facilitated responses to both arms without affecting specific choices of the novel alternative. © 2003 Elsevier B.V. All rights reserved.

Keywords: NMDA receptor; D-Cycloserine; Brightness change; Novelty; Attention and encoding; Memory; Anxiolysis

1. Introduction

A number of reports favor the view that *N*-methyl-D-aspartate (NMDA) receptors are involved in central memory mechanisms. NMDA receptors are abundant in the hippocampus [29,31] and may interact with memory-associated septo-hippocampal cholinergic mechanisms [23]. They are also involved in long-term potentiation, which is believed to be important for learning and memory [11,44]. In recent years, there have been observations of improved learning and retention following systemic and central administration of the partial agonist at the glycine binding site, D-cycloserine (DCS), in normal [17,32,47], brain-lesioned ([33,46] but see [50]), scopolamine-treated ([16,34,40] but see [35]), dizocilpin (MK-801)-treated ([25] but see [36]) and aged animals ([9,48] but see [7]).

Although it is fairly generally agreed that DCS can improve acquisition and working memory, there is less agree-

ment about its enhancement of longer-term retention or consolidation. In this respect, some studies demonstrate improvements [27,37,41] while others show no effect [8,43,44]. And in spite of suggestions based on research with animals that DCS may have potential memory-enhancing properties for sufferers of Alzheimer's disease, most human clinical evidence to date does not support such claims [14,26]. But irrespective of possible clinical implications, the array of often conflicting findings from animal investigations indicates the need for further investigation of the role of the NMDA receptor complex in the processes characterizing normal memory.

Because most animal studies have comprised evaluations of DCS effects on tasks that depend mainly on spatial learning and memory, there is relatively little known about how the compound influences the acquisition and retention of visual information. However, there is some evidence that DCS can attenuate impairments of visual discrimination performance by rats following rearing in isolation [24] or treatment with scopolamine [2]. It has also been shown to improve visual recognition memory in rhesus monkeys, as indicated

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by their performance on a delayed non-matching-to-sample task [30]. By investigating the effects of DCS, the aim of the present study was to further our understanding of how NMDA receptors might be involved in visual recognition requiring memory for the previous spatial location of a maze arm that had since changed in appearance.

The particular model adopted exploits non-deprived rats' tendencies to approach and explore novel stimuli, in the form of a change in brightness between two successive exposures to the arms of a Y maze, without the use of conventional reinforcers [19]. Since the rats are not required to learn responses but to incidentally attend to the brightness characteristics of the maze arms, the principle is not unlike that characterizing studies of memory for stimulus attributes of a training situation [42]. Recent application of this model to the effects of D-glucose has suggested enhancement of memory for change following post-acquisition administration of the compound [21] that is still evident 24 h later, possibly through attenuated forgetting [22]. As pre-acquisition treatment with glucose has also been shown to produce significant responsiveness to brightness change in middle-aged female but not male rats [20], both sexes were investigated throughout.

2. Experiment 1: Pre-acquisition treatment with DCS

The aim of the first experiment was to determine whether or not DCS would affect responsiveness to brightness change when administered before free access to a black and a white Y-maze arm followed by access to two black arms, i.e. pre-acquisition treatment. Typically, untreated rats will enter first the arm that has changed from what it had been in the acquisition phase [13] and continue to re-enter and spend more time in this arm than the unchanged alternative [19]. While any increase in such responsiveness to change following pre-acquisition treatment with DCS could involve enhancement of retention, it might also reflect effects on attentional or encoding processes.

2.1. Materials and methods

2.1.1. Animals

The subjects were 28 Long-Evans hooded rats (14 males, 14 females) approximately 5 months old. They were caged in groups of 3 or 4 same-sexed animals with ad libitum food and water, and kept in conditions of 12 h light/12 h dark and an ambient temperature of 20 ± 1 °C. Testing was carried out during the dark phase of the rats' light/dark cycle. Their care and experimental treatment complied with Parts 5 (Codes of Welfare) and 6 (Use of Animals in Research, Testing, and Teaching) of the New Zealand Animal Welfare Act, 1999. All procedures were approved by the Animal Ethics Committee of the University of Canterbury.

2.1.2. Apparatus

For each rat the apparatus comprised a wooden Y maze with painted aluminum arm inserts described earlier [20].

The arms of the maze were each 45 cm long with an angle of 120° between them, and the stem was 30 cm long. All parts of the apparatus were 10 cm wide and 14 cm high and covered by hinged transparent Perspex lids. During all acquisition and choice trials, a removable black or white insert (consisting of a floor, an end wall and two side walls) was present in each arm. This insert occupied the width, height and the distal 40 cm of the length of the arm. The walls and floor of the stem comprised clear-varnished wood.

The maze was positioned on a 1-m high table so as to ensure that both arms were evenly illuminated by dim fluorescent overhead lighting. The observer was seated behind the stem and concealed from the rat, with a PC computer and keyboard alongside that was used for recording all behavioral observations.

2.1.3. Procedure

Twenty minutes before the acquisition phase of all testing sessions, each rat was intraperitoneally injected (1 ml/kg) with either isotonic saline (0 mg/kg) or 5 or 10 mg/kg DCS (D-4-amino-3-isoxazolidinone, Sigma) dissolved in saline. These doses were within the range shown to enhance learning and memory in rats [28,39,41]. The rat was then placed in the south end of the stem and, for a 6-min acquisition trial, allowed to leave the stem and repeatedly enter the two arms, one of which contained a black insert and the other contained a white insert. It was then removed from the apparatus for approximately 60 s while both arm inserts were replaced with clean black ones to avoid the possibility that subsequent choices of either arm might be determined by odor cues left in the original inserts during the acquisition trial. The rat was then re-introduced into the maze where it was faced with the unchanged stem and two black arms, one of which had changed from white, thereby avoiding any effects on choice behavior of aversions to white arms [19]. Consequently, to recognize the changed arm, the rats also had to remember if the white arm had been on the left or the right hand side. For exactly 60 s (choice trial), the first arm entered by all 4 ft, the total numbers of subsequent entries of each arm and the time spent in them were recorded. As habituation to the novelty of the changed arm dissipates rapidly, there is little evidence of continued responsiveness to brightness change after 1 min of observation [19].

All rats experienced a total of 6 acquisition followed by 6 choice trials. They were administered each of the three DCS levels twice in a non-systematic fashion, with 2 or 3 days intervening between a choice trial and the next acquisition trial. For one of each pair of choice trials, the changed (or novel) arm was on the left and for the other it was on the right.

2.1.4. Data analysis

The first arm entered, the average number of times per day the two arms of the Y maze were entered, the percentage of entries of the novel arm, the average time per day spent in both arms and the percentage of time spent in the

Table 1

Mean (\pm S.E.M.) percent first entries and repeated entries of and time spent in the novel arm following pre-acquisition treatment with three doses of DCS, effects of sex, and results of F -tests for main effects and DCS \times sex interactions

	DCS dose (mg/kg)			$F(2,52)$
	0	5	10	
First entries	41.07 (6.83)	64.29 (6.73) [†]	60.71 (5.37) [†]	4.50**
Repeated entries	45.11 (2.70)	56.28 (3.21) [†]	54.61 (2.22) [†]	6.59**
Time spent	45.08 (3.75)	55.44 (3.82)	60.41 (3.16) [†]	6.64***
	Sex			
	Males	Females	$F(1,26)$	$F(2,52)$ interaction
First entries	53.57 (8.23)	57.15 (6.64)	0.18	1.19
Repeated entries	51.18 (3.42)	52.82 (1.42)	0.20	6.59***
Time spent	53.93 (4.63)	53.35 (2.16)	0.01	3.00*

* $P < 0.05$.

** $P < 0.02$.

*** $P < 0.003$.

[†] Significantly greater ($P < 0.05$) than a chance expectancy of 50%.

novel arm following treatment with each dose of DCS were determined for individual rats. These averages were used in subsequent statistical analyses, except first entries of the novel arm, for which totals were used. All data were subjected to DCS \times sex repeated measures ANOVAs and Scheffe post hoc tests. When appropriate, frequencies of novel arm choice measures were assessed for significance, compared with chance expectancies of 50%, by one-sample t tests.

2.2. Results

2.2.1. Choices of the novel changed arm

The effects of DCS and sex on first entries of the novel arm (expressed as percentages), and per cent entries of and time spent in the novel arm are shown in Table 1.

DCS treatment significantly affected all three measures of novel arm choice. For first entries of the novel arm, this arose from a significantly higher frequency ($P < 0.05$) fol-

lowing treatment with both doses of DCS. However, similar outcomes for the longer term measures of choice are more appropriately interpreted in terms of significant DCS \times sex interactions for both (see Fig. 1).

As shown by one-way ANOVAs for each sex separately, DCS treatment significantly affected repeated entries of the novel arm by male rats ($F(2, 26) = 4.20$, $P < 0.03$), but not the relative time they spent in it ($F(2, 26) = 1.62$, NS). The outcome in the former case was due to a significant increase with 5 but not with 10 mg/kg. However, for the two measures, males' choices of the novel arm exceeded chance expectations after treatment with 5 but not with 10 mg/kg DCS.

The DCS effect was significant for female rats for both repeated entries ($F(2, 26) = 8.98$, $P < 0.002$) and time spent ($F(2, 26) = 12.42$, $P < 0.0002$) because of significantly higher percentages of choice of the novel arm after treatment with 10 mg/kg only (which reached levels greater than expected by chance).

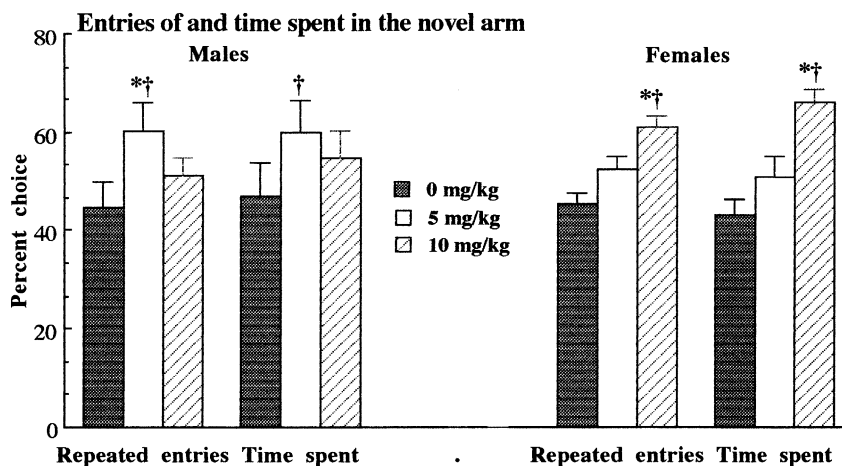


Fig. 1. Mean (\pm S.E.M.) repeated entries of and time spent in the novel arm following pre-acquisition treatment with DCS for males ($n = 14$) and females ($n = 14$) separately. *Significantly different ($P < 0.05$) from vehicle treatment condition. [†]Significantly greater ($P < 0.05$) than a chance expectancy of 50%.

Table 2

Mean (\pm S.E.M.) per day latencies of entering an arm, and repeated entries of and time spent in both arms following pre-acquisition treatment with three doses of DCS, effects of sex, and results of *F*-tests for main effects and DCS \times sex interactions

	DCS dose (mg/kg)			<i>F</i> (2,52)
	0	5	10	
Latencies (s)	19.15 (5.30)	15.54 (4.83)	19.97 (5.46)	0.25
Repeated entries	2.73 (0.17)	2.29 (0.14)	2.86 (0.15)	6.88*
Time spent (s)	31.47 (1.12)	27.98 (1.82)	30.13 (1.10)	1.68
	Sex		<i>F</i> (1,26)	<i>F</i> (2,52) interaction
	Males	Females		
Latencies (s)	27.90 (6.21)	8.54 (0.90)	9.51*	0.01
Repeated entries	2.28 (0.15)	2.98 (0.13)	12.68*	0.25
Time spent (s)	30.41 (1.28)	29.31 (1.04)	0.45	1.61

* $P < 0.003$.

No other interaction or overall sex difference was significant for any of the measures.

2.2.2. Choices of both maze arms

Effects of DCS and sex on latencies to enter an arm, and repeated entries of and time spent in both arms can be seen in Table 2.

Of these three measures, only repeated entries were significantly affected by DCS treatment. This was due to significantly fewer entries being made after treatment with 5 mg/kg DCS than with either 10 mg/kg or vehicle ($P < 0.05$).

Female rats entered one of the arms significantly faster and then repeatedly entered both of them more often than males. No interaction was significant.

2.3. Discussion

Both doses of DCS significantly increased first entries of the novel arm for all rats to levels that indicated significant preferences for this arm. However, the nature of effects of the treatment on longer-term measures of choice depended on the sex of the subjects, thereby justifying investigation of both sexes rather than males only (which still typifies the overwhelming majority of animal studies in this and other related areas). As indicated in Fig. 1, it appears that effects of the compound on responsiveness to novelty for males reflected an inverted U-shaped relationship with maximum increases occurring following administration of 5 mg/kg. However, such an outcome achieved statistical significance in only one case, namely, repeated entries of the novel arm, although, following this effective dose, choices of the arm were beyond chance expectancies for both this and the related time-spent measure. Inverted U-shaped effects of DCS on learning have also been reported [30,32], possibly because of NMDA agonism with lower followed by antagonism with higher doses [3]. On the other hand, for females, maximum increases occurred (in both measures) only after treatment with the highest dose. It is therefore possible that, by achieving with a lower dose a similar outcome to that resulting from a much higher dose for females, contrary to an earlier suggestion [15], males may be more sensitive to

the effects of DCS, at least with respect to novelty choices in the present experimental context.

Of the responses recorded that were not specifically directed towards choices of the novel arm, only repeated entries of both arms were affected by DCS in a U-shaped fashion whereby decreases were observed with 5 but not 10 mg/kg. While this might suggest a dose-dependent depressant effect of the compound on more locomotor activity-dependent choices of both arms (irrespective of their novelty value), from inspection of Tables 1 and 2 and Fig. 1, there is no obvious relationship between these and the novelty choice measures. It is therefore unlikely that, any of the effects of DCS on choices of both arms were responsible for its effects on specific choices of the novel arm.

It may initially appear that pre-acquisition treatment with 5 mg/kg DCS for males and 10 mg/kg for females improved their memory for which maze arm had changed in brightness, especially as responsiveness to change has been regarded by some investigators as a test of recognition memory [10,38]. This would be consistent with a report of improved visual recognition memory in rhesus monkeys following treatment with 320 but not 1000 μ g/kg DCS [26]. However, because the rats in the present study were treated with DCS before their acquisition trials, it is also possible that the effects observed were also (or mainly) due to improved attention and/or encoding rather than to enhanced retention alone, as was concluded for delayed matching-to-position [43]. To rule out effects of the compound on attention and/or encoding, it would therefore be preferable to administer DCS after the acquisition phase but before the change in brightness is introduced. This change in timing of treatment with DCS was the subject of a second experiment.

3. Experiment 2: Post-acquisition treatment with DCS

3.1. Materials and methods

The subjects comprised a further 14 male and 14 female Long-Evans hooded rats, approximately 5.5 months old. They were housed in the same conditions and tested in the

Table 3

Mean (\pm S.E.M.) percent first entries and repeated entries of and time spent in the novel arm following post-acquisition treatment with three doses of DCS, effects of sex, and results of *F*-tests and DCS \times sex interactions

	DCS dose (mg/kg)			<i>F</i> (2,52)
	0	5	10	
First entries	46.43 (6.77)	64.29 (6.73) [†]	62.5 (6.1) [†]	2.59
Repeated entries	46.55 (4.60)	57.04 (2.90) [†]	52.30 (1.62)	2.38
Time spent	51.04 (5.40)	58.34 (4.71) [†]	50.78 (2.90)	0.94
	Sex			
	Males	Females	<i>F</i> (1,26)	<i>F</i> (2,52) interaction
First entries	55.95 (5.69)	59.52 (5.72)	0.20	3.34*
Repeated entries	51.07 (2.64)	52.86 (1.99)	0.29	2.09
Time spent	48.12 (4.24)	58.66 (2.63) [†]	4.46*	0.77

* $P < 0.05$.

[†] Significantly greater ($P < 0.05$) than a chance expectancy of 50%.

same Y maze as for Experiment 1. The procedure was the same as for Experiment 1 except that the rats were treated with saline or DCS (5 or 10 mg/kg) after rather than before their 6-min acquisition trials. Twenty minutes later, each rat was returned to the maze for its choice trial with a changed and an unchanged arm. The method of introducing a brightness change, the behavioral observations, the number of trials and days and the type of data analyses were identical to Experiment 1.

3.2. Results

3.2.1. Choices of the novel changed arm

Effects of DCS and sex on first entries of the novel arm (expressed as percentages), per cent entries of and time spent in the novel arm, and results of ANOVAs are shown in Table 3.

In no case was the DCS main effect significant, although significant choices of the novel arm following treatment occurred in a number of cases. However, there was a significant DCS \times sex interaction for first entries of the novel arm which was due to a significant DCS effect for males ($F(2, 26) = 5.12$, $P < 0.015$), but not for females ($F(2, 26) = 0.75$, NS). As shown in Fig. 2, males entered the novel arm first significantly more often following treatment with 5 mg/kg DCS than after injections with vehicle only. These entries exceeded chance expectations after 5 for males and 10 mg/kg for females.

While females spent significantly more time in the novel arm than males, no other sex difference or interaction (other than that described above) was significant.

3.2.2. Choices of both maze arms

Effects of DCS and sex on latencies to enter an arm, and repeated entries of and time spent in both arms are outlined in Table 4.

The DCS effect was significant in each case. While both doses significantly decreased latencies of entering an arm,

and increased the amount of time subsequently spent in both of them ($P < 0.05$), repeated entries were increased only by 10 mg/kg. Females entered an arm significantly faster and then repeatedly entered both arms more often than males. No interactions were significant.

In view of the significant relationships between DCS treatment and all responses to both arms and the possibility that these might have determined effects of the compound on choices of the novel arm, correlations were computed between each of the three former measures followed by correlations between each of these and each one of the three latter measures (see Table 5).

There were significant negative correlations between latencies of entering an arm and each of the other two measures of responsiveness to both arms. These latter two measures were also significantly positively correlated. No other correlations were significant.

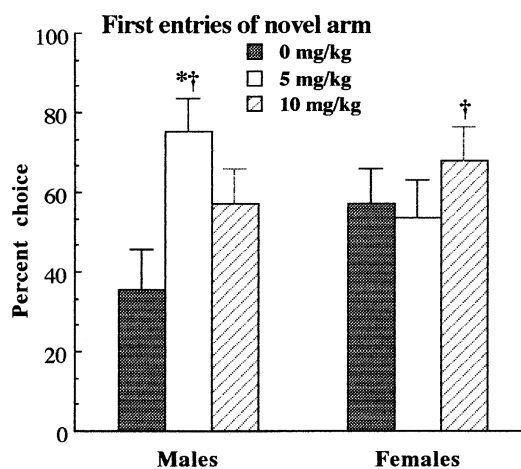


Fig. 2. Mean (\pm S.E.M.) first entries of the novel arm following post-acquisition treatment with DCS for males ($n = 14$) and females ($n = 14$) separately. *Significantly different ($P < 0.05$) from vehicle treatment condition. [†]Significantly greater ($P < 0.05$) than a chance expectancy of 50%.

Table 4

Mean (\pm S.E.M.) per day latencies of entering an arm, and repeated entries of and time spent both arms following post-acquisition treatment with three doses of DCS, effects of sex, and results of *F*-tests for main effects and DCS \times sex interactions

	DCS dose (mg/kg)			<i>F</i> (2,52)
	0	5	10	
Latencies (s)	56.54 (14.50)	22.06 (7.07)	8.77 (1.69)	7.00**
Repeated entries	2.22 (0.24)	2.22 (0.17)	3.43 (0.16)	7.49**
Time spent (s)	22.25 (2.20)	28.14 (2.26)	27.77 (1.26)	3.36*
	Sex			<i>F</i> (2,52) interaction
	Males	Females	<i>F</i> (1,26)	
Latencies (s)	44.59 (8.09)	13.66 (4.23)	11.47**	1.40
Repeated entries	1.86 (0.15)	3.03 (0.17)	28.64***	1.08
Time spent (s)	23.63 (1.90)	28.48 (1.58)	3.87	0.91

* $P < 0.05$.

** $P < 0.003$.

*** $P < 0.0001$.

3.3. Discussion

While there were again some sex-dependent results, they were not as evident as in Experiment 1. Apart from an increase in first entries of the novel arm for males only following treatment with 5 mg/kg DCS, the compound did not significantly affect the other two measures of responsiveness to the change in brightness. However, it should be noted that, contrary to what occurred following treatment with vehicle, first entries of the novel arm significantly exceeded chance for males after 5 mg/kg DCS, and for females after 10 mg/kg. This pattern was similar to that for the longer-term measures in Experiment 1. For both sexes combined, there were also significant above-chance repeated entries of and time spent in the novel arm following treatment with 5 but not 10 mg/kg DCS. It, therefore, seems likely that the lower (but not higher) dose of post-acquisition DCS improved the rats' longer-term ability to distinguish between the unchanged and novel changed arms of the maze, thereby supporting involvement of NMDA receptors in memory as well as attention and/or encoding. However, as the effects were generally less sex-dependent than in Experiment 1, it is possible that the rats' sex may have been less important for determining effects of DCS on

the retention component of responsiveness to novelty than on attention and/or encoding (that would have been more a feature of Experiment 1 than it was of Experiment 2).

But DCS-induced changes in responses to both maze arms, in the form of reduced latencies of entering one of them, increased repeated entries of (for males only) and time spent in them both, suggested the operation of processes in addition to choices of novelty. Even though the rats were not treated with DCS until after their acquisition trials, they nevertheless faced the changed and unchanged arms while still under the influence of the compound. It is therefore possible that increased tendencies to enter (as also occurred for females in Experiment 1) and occupy the arms were due to lowered fear arising from the anxiolytic effects of DCS [3,49]. Locomotor activity (on which the frequency of arm entries and their occupation depended) is inversely related to fear [4]. Also, as fear will decrease the speed of movement from a familiar to a more novel environment [4], latencies to enter an arm in the present study could be construed as involving movement from the familiar stem of the maze to an area that was generally more novel because one of the arms had changed in brightness. Further support for the fear-related basis of these measures is found in the negative correlations between latencies

Table 5

Coefficients (d.f. = 26) for correlations, A, between each of the three measures of responsiveness to both arms and, B, between each measure of responsiveness to both arms and each of the three measures of choice of the novel arm

	Latency of entering an arm	Entries of both arms	Time spent in both arms
(A)			
Latency of entering an arm	–	–0.67**	–0.39*
Entries of both arms	–	–	0.43*
(B)			
Percent entries of novel arm	–0.12	0.10	–0.01
Percent repeated entries of novel arm	–0.18	0.10	0.13
Percent time spent in novel arm	–0.23	0.28	0.33

* $P < 0.05$.

** $P < 0.001$.

and repeated entries of as well as time spent in the two arms.

While anxiolytic effects of DCS may have played a part in determining responses to both arms, they are less likely to have been the only reason for effects of the compound on choices of the novel arm. This is suggested by the lack of significant correlations between any of the responses to both arms and the three measures of novel arm choice.

4. Conclusions

The results of the two experiments taken together suggest that agonism of NMDA receptors via systemic treatment with DCS enhanced retention of information required for the subsequent recognition of a brightness change. It may also have improved attention to and/or encoding (and thus acquisition) of this information. Unfortunately, it is not possible to determine the relative extents to which retention (if any) or attention/encoding were affected in Experiment 1. However, because post-acquisition treatment with DCS was adopted in Experiment 2, the effects observed were more likely to be due to improved retention. So depending on exactly how the results of the two experiments are interpreted, it might be concluded that the study supported earlier work suggesting facilitation of mainly attention or encoding by DCS [45], while also providing some limited support for its memory-enhancing properties [27,37,41].

In addition, the results showed that, while male and female rats differed in their responsiveness to DCS, this difference was more apparent in Experiment 1 where opportunities for the compound's effects on attentional and/or encoding processes were greater. Generally, it would appear that the males may have been more sensitive to DCS than females since they showed increases in choices of the novel arm with the lower of the two doses, whereas females required the higher dose to achieve similar increases. However, tendencies to choose the novel arm following treatment with vehicle alone were not significant in the two experiments for either sex, thereby suggesting that the rats may have forgotten which of the two black arms was previously white. If so, then it is possible that the principal retention-related effect of DCS was to prevent such forgetting (particularly in Experiment 2), as has been concluded for its effects on memory for the contextual attributes of a learning situation [27]. Alternatively, failures to choose the changed arm when treated with saline alone may have reflected a fear-related decrease in preference for novelty.

It is possible that post (but perhaps less so for pre) acquisition treatment with DCS reduced any fear that the rats' may have had of the experimental situation, thereby increasing their willingness to enter and occupy both arms of the apparatus while not interfering with their ability to recognize the changed arm. In both experiments, females entered one of the arms faster and then repeatedly entered them more often than males. The latter result is consistent with observations

of higher levels of locomotion in female rats than in males [5]. However, because male rats are generally regarded as more fearful than females [6,18], these sex differences and the significant negative correlation between both responses plus the inverse relationship between fear and locomotion [4] all add further weight to the assertion that they may have both reflected levels of fear in the rats, and thus anxiolysis [3,49] following DCS treatment.

Although the rats in both experiments had not experienced systematic pre-experimental handling (apart from that necessitated by weekly cage-cleaning from soon after birth until testing), they did not appear unduly fearful before, during or after their times in the apparatus. Nevertheless, it is significant that, contrary to what typifies non-injected animals [13,19], administration of saline alone was followed by a lack of preference for the novel arm shown by both sexes (as outlined in Figs. 1 and 2). Saline injections have been shown to reduce mouse locomotor activity [12] in a manner consistent with heightened fear arising from an aversive experience [4]. And aversive experiences are known to decrease preferences for novelty in rats [1]. Therefore, because of possible aversiveness of the injection procedure (and in spite of the non-significant correlations outlined in Table 5), the addition of DCS may have attenuated any resulting fear thereby leading to significant preferences for the novel arm. However, it should be noted that evidence of DCS-induced anxiolysis in rats, as measured by the fear-potentiated startle reflex, has only been reported for intraperitoneal doses likely to result in NMDA antagonism rather than agonism, namely >30 mg/kg [3]. But on the other hand, anxiety-related behavior observed in the elevated plus maze has been reduced in male rats following intraperitoneal administration of 12 mg/kg DCS [15]. Therefore, while most of the data described in this present paper favor memory enhancement by DCS, anxiolytic effects of the compound cannot be entirely ruled out as possibly contributing to responsiveness to change or other tasks involving recognition of novelty.

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