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D-Cycloserine reverses scopolamine-induced object and place memory deficits in a spontaneous recognition paradigm in rats

Takaaki Ozawa, Kazuo Yamada, Yukio Ichitani*

Institute of Psychology and Behavioral Neuroscience, University of Tsukuba, Tsukuba, Ibaraki 305-8577, Japan

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ABSTRACT

D-Cycloserine (DCS) is a partial agonist of the glutamatergic *N*-methyl-D-aspartate (NMDA) receptor-associated glycine site, and it prevents the amnesic effects of the muscarinic receptor antagonist scopolamine in various memory tests in rodents. In the present study, we tested the hypothesis that DCS has anti-amnesic effects in scopolamine-induced deficits using spontaneous object recognition and place recognition tests. In both tests, scopolamine (0.5 mg/kg, i.p.) was systemically administered 60 min prior to testing, while DCS (7.5, 15, 30 mg/kg, i.p.) was administered 30 min before testing, which consisted of a sample phase (5 min), a delay interval (15 min) and a test phase (2 min). DCS treatment reversed scopolamine-induced deficits in discriminatory behavior during the test phase. However, DCS did not affect decreased object exploration itself or increased thigmotaxis in the open-field arena induced by scopolamine. These results support our hypothesis and suggest differential contributions of glutamatergic-cholinergic system interactions to recognition memory and non-mnemonic exploratory behaviors.

1. Introduction

The *N*-methyl-D-aspartate (NMDA) receptor is an ionotropic glutamate receptor that plays an important role in neural plasticity in the central nerves system. Pharmacological blockade of NMDA receptors impairs induction of long-term potentiation (LTP) in the brain (Harris et al., 1984; Morris, 1989) and disrupts performance in memory tasks in experimental animals (Danysz et al., 1988; Kawabe et al., 1998a; Morris, 1989; Morris et al., 1986). D-Cycloserine (DCS) is an exogenous partial agonist of the NMDA receptor-associated glycine site, which modulates activation of NMDA receptors (Hood et al., 1989; Monahan et al., 1989). DCS has been suggested to be a potential cognitive enhancer. Previous studies have reported that systemic administration of DCS attenuates various memory deficits caused by brain lesions (Myhrer and Paulsen, 1992; Schuster and Schmidt, 1992), chemical substances (Kawabe et al., 1998b; Maurice et al., 1996) and aging (Baxter et al., 1994; Riekkinen et al., 1997; Riekkinen and Riekkinen, 1997; Schwartz et al., 1996). Further, DCS improves learning and/or memory, even in normal animals (Lelong et al., 2001; Monahan et al., 1989; Ozawa et al., 2012; Pussinen and Sirviö, 1999; Quartermain et al., 1994).

Acetylcholine is a key neuromodulator that regulates adaptive neural plasticity and learning behaviors through activation of target receptors, such as nicotinic and muscarinic receptors. Previous studies have shown that treatment with the muscarinic receptor antagonist scopolamine impairs induction of LTP in animal brain (Hirotsu et al., 1989; Watanabe et al., 1995) and disrupts performance in various memory tasks in rodents (Burešová et al., 1986; Ennaceur and Meliani, 1992; Pitsikas, 2007; Rudy, 1996) and humans (Jones et al., 1991; Wesnes et al., 1991). Importantly, hypofunctionality of the cholinergic system has been demonstrated as one of the pathogenic cognitive changes in Alzheimer's disease (AD) (Giacobini, 1990). Many studies have exploited the amnesic effects of scopolamine as a pharmacological model of AD in experimental animals (Beatty et al., 1986; Collerton, 1986; Ebert and Kirch, 1998; Kopelman and Corn, 1988).

Cholinergic system also has some roles in emotional behavior as indicated by the non-cognitive psychiatric manifestations in AD such as increased anxiety. In rodent models, drug-induced behavioral changes in the open-field locomotor activity have been investigated to assess the effect of drugs on the emotion. In particular, it has been shown that the administration of scopolamine increases the amount of time spent in the perimeter in the open-field arena, which is known as thigmotaxic behavior (Sienkiewicz-Jarosz et al., 2000; Thiel et al., 1999). Importantly, this facilitative effect on thigmotaxis has been regarded as an anxiogenic effect of the drug (Simon et al., 1994).

NMDA receptor- and muscarinic receptor-mediated signaling

* Corresponding author at: Institute of Psychology, University of Tsukuba, Tennoudai 1-1-1, Tsukuba, Ibaraki 305-8577, Japan.
E-mail address: y-ichit@human.tsukuba.ac.jp (Y. Ichitani).

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pathways interact with each other. Pharmacological stimulation of muscarinic receptors enhances NMDA-evoked currents in the hippocampus (Lu et al., 1999) and striatum (Calabresi et al., 1998; Pisani et al., 2001). Furthermore, activation of NMDA receptors is necessary for LTP, which is induced by pharmacological activation of muscarinic receptors (Dennis et al., 2016). NMDA also enhances the function of muscarinic receptors by facilitating their phosphorylation (Butcher et al., 2009).

The amnesic effects seen in scopolamine-induced deficits have been used as a platform for drug screening of potential cognitive enhancers. DCS treatment reverses scopolamine-induced learning and memory impairments in rats in multiple memory tasks, including the water maze (Fishkin et al., 1993; Sirviö et al., 1992), T-maze (Fishkin et al., 1993), brightness discrimination test (Andersen et al., 2002), three-panel runway working memory task (Ohno and Watanabe, 1996), passive avoidance (Zajaczkowski and Danysz, 1997) and social transmission of food preference (Portero-Tresserra et al., 2013, 2014). Supporting these promnesic effects on behavior, DCS treatment also prevents suppression of LTP induced by scopolamine in the hippocampus (Portero-Tresserra et al., 2014). On the other hand, it has never been tested whether DCS has any effect on the anxiogenic effect of scopolamine.

Recognition memory is an essential cognitive function for animals to discriminate a stimulus that has been previously experienced from one that is new. To assess recognition memory in rats, there are at least two types of widely used spontaneous recognition tests, the spontaneous “object” recognition (SOR) test (Ennaceur and Delacour, 1988) and the spontaneous “place” recognition (SPR) test (Ennaceur et al., 1997; Ozawa et al., 2011), wherein the object memory or the object location memory is tested, respectively. These spontaneous recognition tests rely on the animals' innate tendency to respond more toward the novel stimulus compared to the familiar one. They have important advantages that we can evaluate recognition memory without depending on animals' motivation to seek food reward or to escape from aversive stimulus, and also without animals' rule learning, both themselves can be affected by the pharmacological manipulations. Furthermore, simultaneously but separately, we can also evaluate the drug effect on exploratory behavior by assessing animals' behavior in the open-field in the SOR and SPR tests.

Previous studies revealed the activation of muscarinic receptors is necessary for the successful object and object-location discrimination in the SOR and SPR tests (Assini et al., 2009; Ennaceur and Meliani, 1992; Pitsikas, 2007). However, it remains unknown whether DCS attenuates scopolamine-induced recognition memory deficits in rats. In the present study, by taking advantages of the SOR and SPR tests, we tested the hypothesis that DCS prevents scopolamine-induced object and spatial memory deficits. We also investigated whether DCS reverses scopolamine-induced changes in open-field exploratory behavior in these memory tests.

2. Materials and methods

2.1. Subjects

Twenty-three male Wistar–Imamichi rats (8 weeks old) were used in this study. Mean body weight at the start of experiments was 279 g. Rats were housed in individual cages on a 12:12 h light–dark cycle (light on: 0800–2000) with free access to water and food throughout the experiment. Animal experiments were approved by the University of Tsukuba Committee on Animal Research. All efforts were made to minimize the number of animals used and their suffering.

2.2. Drugs

Scopolamine hydrobromide (Sigma, MO) was diluted in saline (SAL) to a final concentration of 0.5 mg/ml. This dose of scopolamine was chosen based on a previous report (Hiraga and Iwasaki, 1984). D-Cycloserine (Sigma, MO) was freshly dissolved in SAL at the concentration of 7.5, 15 or 30 mg/ml on the day of drug testing. These three doses were selected according to our previous study which showed facilitative effect of DCS on spatial recognition memory (Ozawa et al., 2012).

2.3. Spontaneous recognition tests

2.3.1. Apparatus

An open field arena (90 × 90 × 45 cm) with black walls and a gray floor composed of polyvinyl chloride was used. Notably, a white-black vertically striped pattern was placed on one of the walls as an absolute spatial cue in the SPR test but not in the SOR test. The objects used in the experiments included black and white triangular cast metals, black and white cylinders of cast metal, cans of juice, bottles of coffee, soap cases, glass bottles and yellow rubber ducks. All objects were heavy enough by themselves or were fixed to a heavy metal plate so that the rat was unable to move them. A video camera was suspended above the arena, and images were projected to a monitor to allow the experimenter to observe the animals' behavior.

2.3.2. Test procedures

Rats received 5 min of handling and were allowed 10 min habituation to the apparatus for 5 days. After habituation, the SOR and SPR tests were conducted. In both tests, one trial consisted of a sample phase (5 min) and a test phase (2 min). These two phases were separated by a delay interval (15 min) (Fig. 1A and Fig. 3A). In the sample phase, two identical objects were placed diagonally in the arena (the center of each object was 22.5 cm from the adjacent two walls), and a rat was released into the center of the arena and allowed to freely explore the open-field arena. The rat was then removed from the arena and returned to its home cage for the delay interval. After the delay, the rat was returned to the arena, constituting the test phase. In the SOR test, two objects were placed at the same position as in the sample phase, but only one object was identical to that in the sample phase (familiar object, object F) while the other was a novel object (object N) in the test phase (Fig. 1A). Different object pairs were used for each drug test, and the objects assigned as novel objects were counterbalanced across the tests. In contrast, in the SPR test, one object was placed in the same original position as in the sample phase (object F), while the other was moved to a different position (object N) 30 cm away from object F and 22.5 cm from a sidewall (two locations were possible) (Fig. 3A). Different pairs of objects and different locations in the arena were randomly used in each drug test, and possible novel positions were also counterbalanced across the tests. The floor of the arena and the objects were cleaned using 70% ethanol after sample and test phases. In each phase, we measured the time rats spent exploring the objects. Exploration which is defined as the rat directing its nose toward each object within a distance of 2 cm was manually scored by the experimenter blind to the drug condition of the animals. In addition, locomotor activity and time within the perimeter were analyzed by the Image OF program for the open field test (O'Hara & Co., Ltd., Tokyo) based on the public domain NIH Image program (developed at the U.S. National Institutes of Health). The perimeter in the arena was defined as the area within 15 cm from the sidewalls.

2.3.3. Drug tests

All rats were assigned either to the SOR ($n = 11$) or SPR test ($n = 12$). In drug tests, the effect of combinational intraperitoneal administration of scopolamine (0.5 mg/kg) and DCS (7.5, 15 or 30 mg/kg) on SOR and SPR test performance was evaluated. SAL or scopolamine was injected 60 min before the sample phase, and subsequently, SAL or DCS was administered 30 min before the sample phase in both SOR and SPR tests (Fig. 1A and Fig. 3A). All combinations of drug treatments were tested in a random order using a within-subject design. There was a minimum interval of 48 h between drug tests.

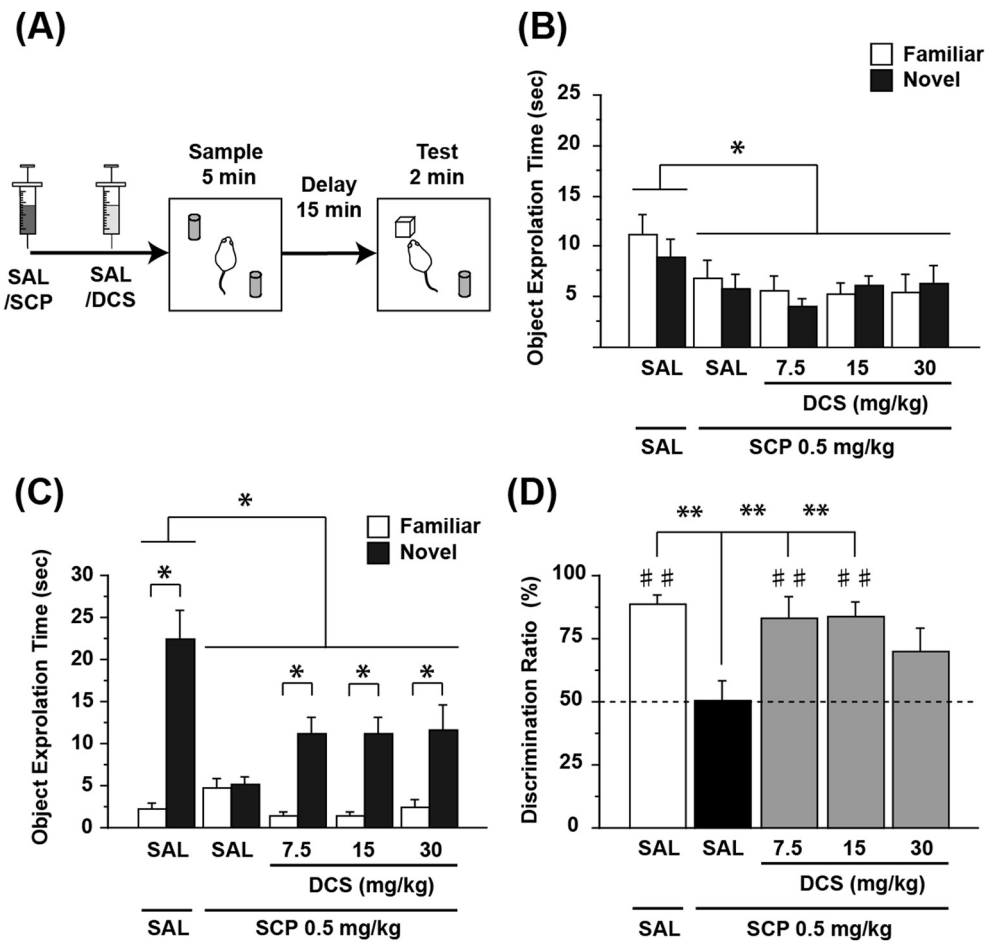

Fig. 1. Effects of systemic administration of scopolamine (SCP) and D-cycloserine (DCS) on performance in the spontaneous object recognition (SOR) test. (A) Schematic illustration of the SOR test, consisting of the sample phase, the delay interval and the test phase. Animals were allowed to explore two identical objects during the sample phase. After the delay interval, animals were returned to the same open-field arena where one of the objects was replaced with a novel object (test phase). SCP (0.5 mg/kg) or saline (SAL) was systemically injected 60 min before the trial, while DCS (7.5, 15, 30 mg/kg) or SAL was administered 30 min before the trial. (B) Time spent exploring each object during the sample phase. "Familiar" indicates the object that is the same, while "Novel" indicates the object that is replaced with a novel object during the test phase. (C) Time spent exploring each object during the test phase. (D) Discrimination ratios, which were calculated by dividing the amount of exploration for the novel object by the total amount of object exploration in the test phase. Data are presented as the mean ± SEM. $*p < 0.05$, $**p < 0.01$. $\#\#p < 0.01$ vs. theoretical chance (50%).

2.4. Statistical analysis

In sample and test phases, the amount of time spent exploring each object was analyzed by two-way repeated measures ANOVA (object × drug) followed by post hoc comparisons using a Bonferroni comparison ($p < 0.05$). In the test phase, to assess discrimination, a discrimination ratio (DR) was calculated by dividing the amount of exploration for the object N by the total amount of exploration for both objects (object F + N). DRs were analyzed by one-way repeated measures ANOVA. Further, each DR was compared to the theoretical chance level (50%) using a one-sample *t*-test. Distance traveled and the amount of time spent within the perimeter during the sample and test phases were also analyzed by one-way repeated measures ANOVA. All statistical analyses were performed using SPSS Statistics (IBM).

3. Results

3.1. Spontaneous object recognition test

Results of the SOR test are shown in Fig. 1B–D. In Fig. 1B, the exploration time spent on each object during the sample phase is shown. Scopolamine generally decreased exploratory behavior of objects in the sample phase. According to two-way ANOVA, there was a significant main effect of drug [$F(4, 40) = 4.47$, $p < 0.01$]. The subsequent post hoc test revealed that exploration time under the SAL + SAL condition was greater than under all other conditions ($p < 0.05$).

The time spent exploring each object during the test phase is shown in Fig. 1C. DCS treatment reversed scopolamine-induced impairment in novel object preference. However, it did not affect the decrease in total object exploration time induced by scopolamine. According to two-way ANOVA, there were significant main effects of both the drug [$F(4, 40) = 6.77$, $p < 0.01$] and object [$F(1, 40) = 54.36$, $p < 0.01$]. The post hoc test revealed that total exploration time of both objects under all scopolamine-treated conditions (SCP + SAL or SCP + DCS) was lower than under SAL + SAL ($p < 0.05$). For the main effect of object, exploration time of the novel object was greater than for the familiar object ($p < 0.05$). The interaction between the effects of drug and object was also significant [$F(4, 40) = 8.07$, $p < 0.01$]. Subsequent post hoc test revealed that exploration time of the novel object was greater than for the familiar object under all conditions, except for SCP + SAL ($p < 0.05$).

DRs in the test phase are shown in Fig. 1D. DCS reversed scopolamine-induced impairment in novel object preference. According to one-way ANOVA, there was a significant main effect of drug [$F(4, 40) = 3.84$, $p < 0.01$]. Post hoc test revealed that DRs under SAL + SAL and SCP + DCS 7.5, 15 mg/kg conditions were greater than for SCP + SAL ($p < 0.01$). Moreover, one-sample *t*-test revealed that DRs under SAL + SAL [$t(10) = 11.32$, $p < 0.01$], SCP + DCS 7.5 mg/kg [$t(10) = 3.79$, $p < 0.01$] and SCP + DCS 15 mg/kg [$t(10) = 6.15$, $p < 0.01$] conditions were higher than chance level.

Total distance traveled (Fig. 2A) and time spent in the perimeter (Fig. 2B) during the sample and test phases are shown. Neither scopolamine nor DCS changed total distance traveled. In contrast, scopolamine increased the amount of time spent in the perimeter, and administration of DCS did not affect this increase. One-way ANOVA revealed that there were no effects of the drug on total distance traveled in either phase. However, a significant effect of the drug on time spent in the perimeter was observed in both sample and test phases [$F(4, 40) = 8.40$, $p < 0.01$; $F(4, 40) = 20.10$, $p < 0.01$, respectively]. Subsequent post hoc test revealed that regardless of DCS treatment,

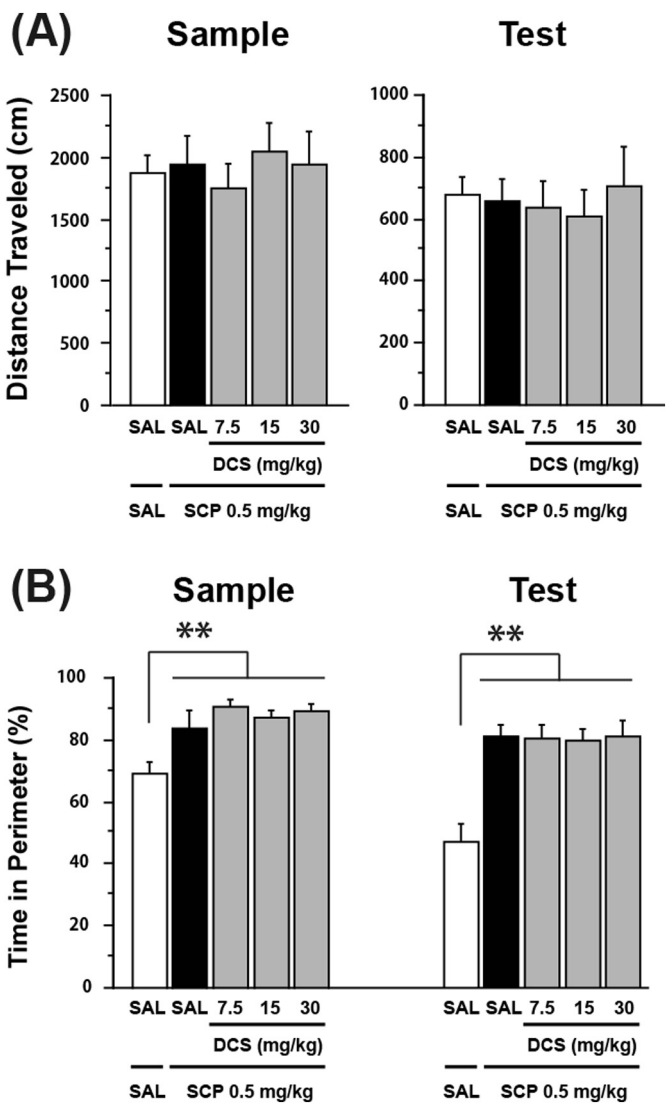

Fig. 2. Effects of systemic administration of scopolamine (SCP) and D-cycloserine (DCS) on non-mnemonic exploratory behaviors in the spontaneous object recognition (SOR) test. (A) Total distance traveled during the sample phase (left) and the test phase (right). (B) Percentage of time spent in the perimeter during the sample phase (left) and the test phase (right). Data are presented as the mean ± SEM. $^{**}p < 0.01$.

scopolamine treatment increased time spent in the perimeter for both sample and test phases ($p < 0.01$).

Since scopolamine decreased the total amount of close and active object exploration in the sample phase, which can be a determinant factor of object recognition performance in the test phase (Albasser et al., 2009; but see Gaskin et al., 2010), we investigated whether object exploration in the sample phase can predict discriminatory performance in the test phase in the present study. However, we did not find the correlations between the object exploration time in the sample phase and the DR score in the test phase in any drug conditions ($-0.119 < r < 0.442$, $p > 0.1$).

3.2. Spontaneous place recognition test

Results of the SPR test are shown in Fig. 3B–D. In Fig. 3B, the exploration time for each object during the sample phase is shown. Scopolamine decreased exploratory behavior of objects during the sample phase. According to two-way ANOVA, there was a significant main effect of drug [$F(4, 44) = 7.17$, $p < 0.01$], and subsequent post hoc test revealed that exploration time under the SAL + SAL condition was greater than under all the other conditions ($p < 0.05$).

Exploration time for each object in the test phase is shown in Fig. 3C. Administration of DCS reversed scopolamine-induced reductions in novel place object preference. However, it did not reverse the decreased total object exploration time induced by scopolamine. According to two-way ANOVA, there were significant main effects of the drug [$F(4, 44) = 11.91$, $p < 0.01$] and object [$F(1, 44) = 16.00$, $p < 0.01$]. Post hoc test revealed that total exploration time for both objects under all scopolamine-treated conditions (SCP + SAL or SCP + DCS) was decreased compared to the SAL + SAL condition ($p < 0.05$). For the main effect of object, exploration time of the novel place object was greater than that for a familiar object ($p < 0.05$). The interaction between the effects of drug and object was also significant [$F(4, 44) = 5.96$, $p < 0.01$]. Subsequent post hoc test revealed that exploration of novel place object was greater than for familiar place object under SAL + SAL, SCP + DCS 15 mg/kg and SCP + DCS 30 mg/kg conditions ($p < 0.05$).

DRs during the test phase are shown in Fig. 3D. In line with results observed for exploration time analysis, scopolamine decreased preference for the novel place object, which was reversed by DCS administration. According to one-way ANOVA, there was a significant main effect of drug [$F(4, 44) = 3.43$, $p < 0.05$], and the post hoc test revealed that DRs under SAL + SAL, SCP + DCS 15, and 30 mg/kg conditions were greater than under the SCP + SAL condition ($p < 0.01$). It was also observed that DRs under SAL + SAL [$t(11) = 4.07$, $p < 0.01$] and SCP + DCS 15 mg/kg [$t(11) = 5.73$, $p < 0.01$] conditions were higher than chance level.

Distance traveled (Fig. 4A) and time spent in the perimeter (Fig. 4B) in sample and test phases are shown. One-way ANOVA revealed that there were no effects of the drug on total distance traveled during each phase. However, similar to results from the SOR test, a significant effect of the drug on time spent in the perimeter was found in both sample and test phases [$F(4, 44) = 7.03$, $p < 0.01$; $F(4, 44) = 10.27$, $p < 0.01$, respectively]. Subsequent post hoc test revealed that scopolamine treatment increased time spent in the perimeter in both sample and test phases ($p < 0.01$), regardless of DCS treatment.

We investigated whether the close and active object exploration in the sample phase can predict discriminatory performance in the test phase. However, we did not find the correlations between the object exploration time in the sample phase and the DR in the test phase in any drug conditions ($-0.41 < r < 0.153$, $p > 0.1$).

4. Discussion

In the present study, systemic administrations of DCS reversed scopolamine-induced deficits in preferential exploratory behavior toward a novel object in the SOR and toward a novel place object in the SPR test. In contrast, DCS did not affect the decrease of object exploration itself or the increase of thigmotaxis in the open-field arena induced by scopolamine in either test.

DCS has been shown to ameliorate scopolamine-induced memory impairment in various memory tasks (Andersen et al., 2002; Fishkin et al., 1993; Ohno and Watanabe, 1996; Portero-Tresserra et al., 2013, 2014; Sirviö et al., 1992; Zajaczkowski and Danysz, 1997). However, the effect of DCS on recognition memory has never been tested. Herein, we demonstrated for the first time an anti-amnesic effect of DCS in scopolamine-induced recognition memory deficits using a spontaneous recognition paradigm. The SOR and SPR tests assess object memory and spatial memory, respectively. To the best of our knowledge, the promnesic effect of DCS on scopolamine-induced memory deficits of object memory has never been reported. In contrast, regarding spatial memory, the anti-amnesic effect of DCS in scopolamine-induced deficits has been previously reported in the water maze task (Fishkin et al., 1993; Puumala et al., 1998; Sirviö et al., 1992). Our results for the SPR test strongly support the anti-amnesic effect of DCS in scopolamine-

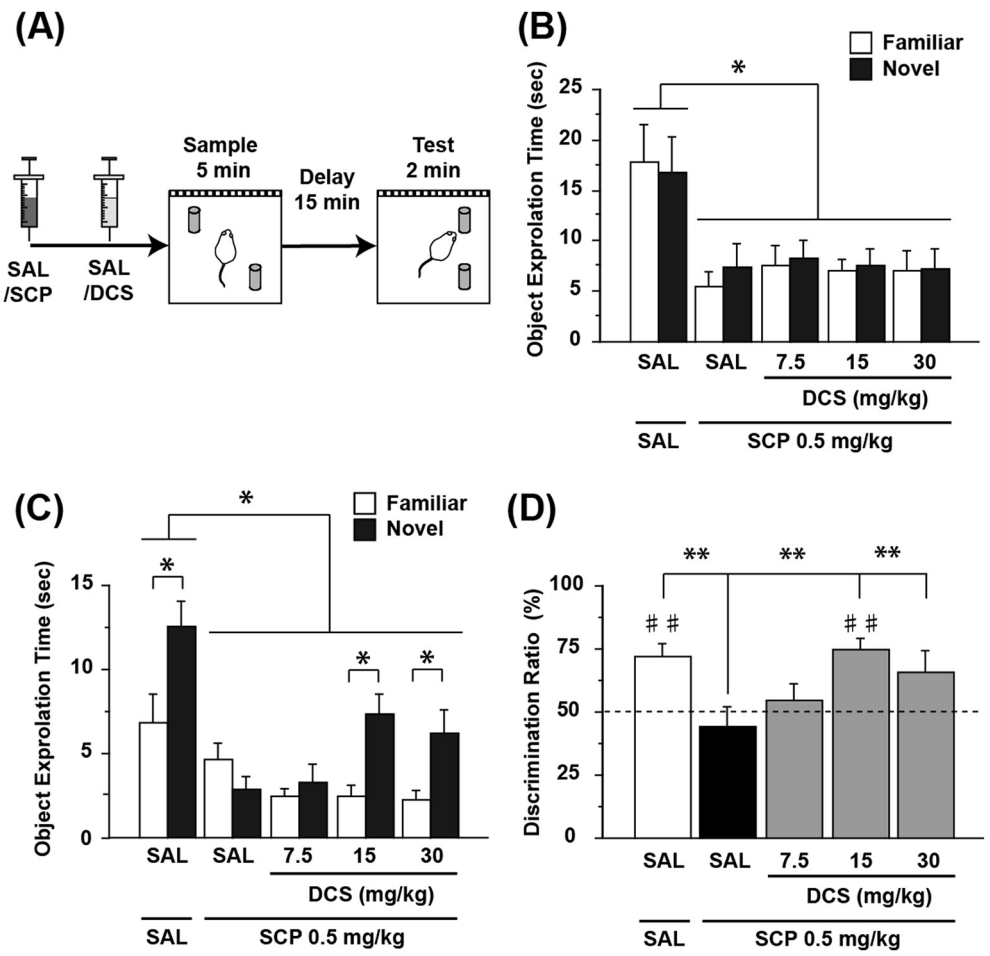

Fig. 3. Effects of systemic administration of scopolamine (SCP) and D-cycloserine (DCS) on performance in the spontaneous place recognition (SPR) test. (A) Schematic illustration of the SPR test, consisting of the sample phase, the delay interval and the test phase. In the SPR test, a white–black striped pattern was put on a sidewall as an absolute spatial cue. Two identical objects were used throughout the trial. Animals were allowed to explore two objects during the sample phase. After a delay interval in the home cage, animals were returned to the same open-field arena where one of the objects had been moved to a novel position (test phase). SCP (0.5 mg/kg) or saline (SAL) was systemically injected 60 min before the trial, while DCS (7.5, 15, or 30 mg/kg) or SAL was administered 30 min before the trial. (B) Time spent exploring each object during the sample phase. "Familiar" indicates the object that is in the same position, and "Novel" indicates the object that is moved to a novel position during the test phase. (C) Time spent exploring each object during the test phase. (D) Discrimination ratios, which were calculated by dividing the amount of exploration for the novel place object by the total amount of object exploration in the test phase. Data are presented as the mean ± SEM. $*p < 0.05$, $**p < 0.01$. $\#\#p < 0.01$ vs. theoretical chance (50%).

induced spatial memory deficits and further suggest its ameliorative effect extends to deficits in spatial recognition memory, which is evaluated independently from the use of aversive/appetitive reinforcers, animals' ability for rule learning and spatial navigation.

We selected three doses of DCS (7.5–30 mg/kg) to investigate the effects on scopolamine-induced amnesia, and found that the dose of 15 mg/kg was consistently effective. This suggests that DCS reverses scopolamine-induced amnesia according to a reversed U-shaped dose-response curve with maximum effect at 15 mg/kg. This is consistent with our previous study where the same doses of DCS were tested in amnesia caused by longer delay interval (24 h) in normal animals (Ozawa et al., 2012). The similar reversed U-shaped response curve of DCS has also been shown in other previous studies (Monahan et al., 1989; Quartermain et al., 1994). In the present study, we found that the same tendency of dose–response relationship could be observed also in scopolamine-induced amnesia suggesting the importance of dose selection of DCS in a possible clinical practice.

Our results suggest that an interaction between NMDA and muscarinic receptor-mediated neurotransmission serves an important role in object and place recognition memory. Although many previous studies have revealed the significance of this interaction, it is still unclear how cholinergic and glutamatergic systems interact to execute recognition memory-guided behavior. As described above, the current study and previous studies have shown the anti-amnesic effect of DCS in scopolamine-induced memory deficits (Andersen et al., 2002; Fishkin et al., 1993; Ohno and Watanabe, 1996; Puumala et al., 1998; Sirviö et al., 1992; Zajaczkowski and Danysz, 1997). Furthermore, the promnesic effect of an acetylcholinesterase (AChE) inhibitor was decreased by administration of an NMDA receptor antagonist (Jafari-Sabet, 2006a, 2006b). These results support the possibility that the glutamatergic system functions downstream of the cholinergic system. However, some previous studies do not necessarily support, or they even oppose, this idea. First, DCS has been reported fail to ameliorate scopolamine-induced memory impairment (Pitkänen et al., 1995; Rupniak et al., 1992). Second, AChE inhibitors reverse NMDA receptor antagonist-induced memory deficits (Csernansky et al., 2005; Patel et al., 1998; Walker and Gold, 1992). Considering these studies tested the effect of drugs with systemic injection, it might be better to investigate the mechanism of cholinergic and glutamatergic interaction by focusing on more specific brain areas.

The perirhinal cortex and hippocampus are considered functionally dissociated regarding recognition memories, that is, each brain region has a dominant role in each of object and spatial memory. Excitotoxic lesions of the perirhinal cortex impaired discriminatory behavior in the SOR test (Ennaceur et al., 1997; Mumby et al., 2002; Winters et al., 2004), while that of the hippocampus impaired novelty discrimination in the SPR test (Barker and Warburton, 2011). Importantly, hippocampal lesions did not necessarily disrupt performance in the SOR test (Barker and Warburton, 2011; Forwood et al., 2005; Mumby et al., 2002; Winters et al., 2004; but see Clark et al., 2000). In line with these, previous pharmacological studies also showed that the infusion of NMDA receptor antagonist into the perirhinal cortex impaired object recognition (Abe et al., 2004), while the hippocampal infusion disrupted spatial recognition without affecting object recognition memory (Yamada et al., 2017). Furthermore, scopolamine infusion into the perirhinal cortex and hippocampus impaired the discriminatory behavior in each of SOR test (Abe et al., 2004) and multi-object spatial novelty detection test (Hunsaker et al., 2007), respectively. Based on this evidence, it is possible that NMDA and muscarinic receptors' signaling are locally interacting each other to regulate object and spatial

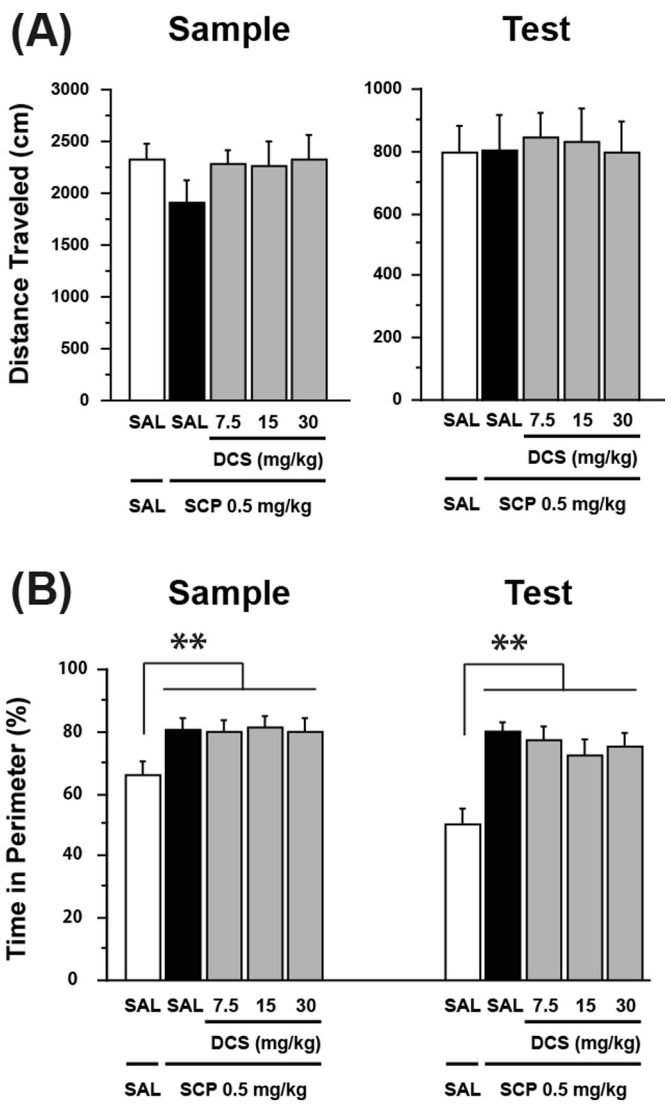

Fig. 4. Effects of systemic administration of scopolamine (SCP) and D-cycloserine (DCS) on non-mnemonic exploratory behaviors in the spontaneous place recognition (SPR) test. (A) Total distance traveled during the sample phase (left) and the test phase (right). (B) Percentage of time spent in the perimeter during the sample phase (left) and the test phase (right). Data are presented as the mean ± SEM. $^{**}p < 0.01$.

recognition memory in the same neuronal population in specific brain areas such as the perirhinal cortex and hippocampus.

On the other hand, it has also been known that glutamate affects recognition memories by regulating the activity of cholinergic projection neurons or local cholinergic release. Indeed, in-vivo quantitative analysis of acetylcholine revealed that cortical or hippocampal acetylcholine release is induced by the activation of glutamatergic receptors in the nucleus basalis and medial septum/vertical limb of the diagonal band which have a lot of cholinergic projection neurons (Giovannini et al., 1997; Moor et al., 1996). Furthermore, it has also been shown that bath application of NMDA enhances acetylcholine level in the cortical slice (Ulus et al., 1992). Given these results, it might be possible that DCS increases acetylcholine release in the perirhinal cortex or hippocampus to the extent which is enough to overcome pharmacological antagonism of muscarinic receptors to reverse scopolamine-induced amnesia in the present study.

Cholinergic system can play roles in object recognition in the SOR and SPR by regulating the attentional processes rather than directly modulating learning and memory. Previous studies showed that lesions of the nucleus basalis impaired performance in various visual attention tasks (McGaughy et al., 2002; Voytko et al., 1994). Optogenetic activation of cholinergic neurons in the nucleus basalis improved animals' visual discrimination (Pinto et al., 2013). Regarding target brain regions, it has been reported that increase of cortical acetylcholine level is correlated with the occurrence of behavioral immediate response to the rewarding cue (Parikh et al., 2007), and that behaviorally related neural activities in the cortex, such as the medial prefrontal cortex and primary visual cortex, are regulated by the cholinergic mechanisms (Gill et al., 2000; Herrero et al., 2008) suggesting the cholinergic projection to the cortex is particularly important. Considering these cholinergic control of attention, in this study, DCS might have increased the acetylcholine release in the cortex to attenuate animals' attentional deficit caused by scopolamine to recover the object recognition. Further studies are needed to reveal the possible effect of DCS on the scopolamine-induced attentional dysfunction.

In the present study, systemic administration of scopolamine also changed exploratory behaviors both in SOR and SPR tests. First, scopolamine decreased object exploration time in both sample and test phases, suggesting that muscarinic-receptor blockade decreased curiosity or even induced phobia for the objects in the arena. Until now, no previous studies examining the effect of scopolamine on performance in the SOR and SPR tests have reported this effect (Pitsikas, 2007; Pitsikas et al., 2001). One possible reason for scopolamine's effect in our study is that the dosage used in our experiment (0.5 mg/kg) was relatively higher than that used in previous studies (0.2 mg/kg). Regarding reduced object exploration time, it should be emphasized that close and active exploration of objects during the sample phase does not necessarily predict animals' discriminatory behavior during the test phase of the SOR and SPR tests. In the SOR test, both positive correlations and no correlation between object exploration time in the sample phase and discrimination score in the test phase have been reported (Albasser et al., 2009; Gaskin et al., 2010), while only no correlation was reported for the SPR test (Ozawa et al., 2011). Actually, we did not find correlations between the object exploration time in the sample phase and the DR in the test phase in any drug condition. Next, scopolamine treatment increased the amount of time spent in the perimeter in the open-field arena, which is known as thigmotaxic behavior, suggesting its anxiogenic effect. Similar to our results, the pro-thigmotaxis effect of scopolamine in the open-field has been observed in previous studies (Sienkiewicz-Jarosz et al., 2000; Thiel et al., 1999). Importantly, in the present study, DCS did not attenuate the abovementioned scopolamine-induced changes in these exploratory behaviors, suggesting that unlike recognition memory, the interaction between NMDA and muscarinic receptor-mediated neurotransmission is not important for non-mnemonic exploratory behavior or emotional behavior.

In addition to thigmotaxis, scopolamine is known to induce hyperlocomotion in the open-field test (Sienkiewicz-Jarosz et al., 2000; Thiel et al., 1999), although this effect was not clearly observed in the present study. Notably, it was shown that familiarization to the experimental room decreased the facilitative effect of scopolamine on locomotor activity in the open-field (Hughes et al., 2004). This indicates the possibility that we did not observe scopolamine-induced hyperlocomotion because rats had already been habituated to the apparatus before drug tests using scopolamine were performed.

5. Conclusion

In the present study, we demonstrated that systemic administration of DCS reverses scopolamine-induced impairment in novelty discrimination in both SOR and SPR tests. However, DCS did not change decreased object exploration itself nor did it alter increased thigmotaxis induced by scopolamine. These results show that DCS improves impairments of object and spatial recognition, but not of non-mnemonic behavior induced by cholinergic hypofunction. This suggests differential contributions of the glutamatergic-cholinergic system interaction

to recognition memory and non-mnemonic exploratory behaviors. Altogether, our results support the possible clinical application of DCS to improve memory deficits in humans caused by cholinergic dysfunction.

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