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ORIGINAL INVESTIGATION

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Effect of intra-accumbens dopamine receptor agents on reactivity to spatial and non-spatial changes in mice

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Abstract *Rationale:* Some evidence suggests an involvement of nucleus accumbens in spatial learning. However, it is controversial whether the mesoaccumbens dopaminergic pathways play a specific role in the acquisition of spatial information. *Objective:* The goal of these experiments was to investigate the effect of dopaminergic manipulations in the nucleus accumbens on a non-associative task designed to estimate the ability to encode/transmit spatial and non-spatial information. *Methods:* The effects of focal administrations of the D1 and D2 dopamine receptor antagonists, SCH 23390 (6.25, 12.5, 50 ng/side) and sulpiride (12.5, 50, 100 ng/side), respectively, and dopamine (DA; 1.25 and 2.5 µg/side) into the nucleus accumbens were studied on reactivity to spatial and non-spatial changes in an open field with objects. *Results:* Both SCH 23390 and sulpiride impaired reactivity to spatial change. However, several differences were found in the effects induced by the two DA antagonists. SCH 23390 did not affect locomotor activity and only slightly impaired exploration of the novel object. On the contrary, the D2 antagonist, induced a general, dose-dependent, impairment on all variables measured. Local administration of DA increased locomotor activity, but did not affect reactivity to spatial and non-spatial changes. *Conclusions:* These results demonstrate a facilitatory role of mesoaccumbens dopamine in the acquisition of spatial information. Moreover, they suggest that nucleus accumbens D1 DA receptors, play a more selective role in the modulation of spatial learning than accumbens D2 DA receptors.

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Introduction

We have recently demonstrated that systemic administration of dopamine (DA) receptor antagonists impairs detection of a spatial novelty in the open field. On the contrary, impaired reaction to object novelty was observed after systemic administrations of direct or indirect DA agonists (Adriani et al. 2000). On the basis of these results we suggested a facilitatory role of DA transmission on spatial learning and an inhibitory effect on novelty exploration (Adriani et al. 2000). The nucleus accumbens together with the dorsal striatum is one of the major projection areas of the dopaminergic system. Systemic administration studies, however, do not allow an assessment of the brain areas responsible for the observed effects. This study was designed in order to investigate the possible involvement of nucleus accumbens DA transmission in modulating reactivity to spatial and non-spatial novelty.

The nucleus accumbens receives dense glutamatergic afferents from the hippocampus and other brain areas involved in spatial learning (Beckstead 1979; de Bruin et al. 1997; Granon et al. 1996; Groenewegen et al. 1987; Kelley and Domesick 1982; Kolb et al. 1982; McDaniel et al. 1994; Morris et al. 1982; Poucet 1989; Save et al. 1992a,b; Thinus-Blanc et al. 1996). On the basis of its neuroanatomical connections, it has been suggested that this structure acts as an interface between the limbic and the motor system in goal-directed behavior (Mogenson et al. 1980). In this view, spatial information would be relayed to the nucleus accumbens through glutamatergic transmission to modulate motor responses or response selection (Pennartz et al. 1994). Some evidence has pointed to this structure as playing a relevant role in spatial learning tasks (Adriani et al. 1996; Cools et al. 1992; Floresco et al. 1996; Ploeger et al. 1994; Seamans and Phillips 1994; Setlow 1997; Taghzouti et al. 1985). For

example, electrolytic (Sutherland and Rodriguez 1989) and, to a lesser extent, ibotenic acid lesions (Annett et al. 1989) impair performance in the Morris water maze. Moreover, glutamate receptor blockade in this structure has been demonstrated to affect response in different spatial learning tasks (Maldonado-Irizarry and Kelley 1995; Sargolini et al. 1999; Shacter et al. 1989; Usiello et al. 1998).

Although the involvement of glutamatergic transmission in the nucleus accumbens in spatial learning has been supported by a number of experimental findings, there are few and contradictory results concerning possible involvement of DA transmission in spatial learning. Thus, on the basis of lesion studies a facilitatory role of DA transmission has been demonstrated in the Morris water maze (Ploeger et al. 1992, 1994; Whishaw and Dunnett 1985). However, it has been suggested that it could be due to a non-specific effect on motor or motivational processes rather than a selective effect on spatial learning (Hagan et al. 1983). Similar conclusions have been drawn from pharmacological studies (Roullet et al. 1996). On the other hand, other studies investigating the effects of lesions or pharmacological manipulations of DA receptors in the accumbens have suggested a more specific role of DA transmission in these abilities (Ploeger et al. 1994; Setlow and McGaugh 1998).

The DA system probably plays some role in the behavioral response to novelty (for a review, see Bardo et al. 1996). Animals are both attracted and aroused by the discovery of novelty (Bardo et al. 1989, 1990; Laviola and Adriani 1998; Misslin and Ropartz 1981a) and by the rearrangement of familiar objects (Wilz and Bolton 1971), as well as by novel objects introduced in a familiar context (Misslin and Ropartz 1981b). Decreased novelty exploration follows in response to systemic administration of a DA agonist (Adriani et al. 2000; Misslin and Ropartz 1981a), this effect being blocked by neuroleptics (Misslin et al. 1984). The involvement of nucleus accumbens DA in novelty response has been demonstrated by different groups. For example, entering a novel environment has been associated with a transient DA release in the nucleus accumbens (Rebec et al. 1997a,b). Furthermore, 6-OHDA-induced lesions of the nucleus accumbens interfere with novelty-induced exploration (Fink and Smith 1980; Pierce et al. 1990).

DA is well known to mediate the reinforcing properties of natural rewards as well as of drugs of abuse (for review see Robbins and Everitt 1996). For this reason, in order to investigate the possible involvement of DA receptors located in the nucleus accumbens in spatial learning, we have opted for a non-associative task where no explicit reward was present. This task consists of placing mice in an open field containing five objects and, after three sessions of habituation, examining their reactivity to object displacement (spatial novelty) and object substitution (object novelty). Control animals usually show an increased exploration of the displaced and substituted objects, this response being usually interpreted as an index of the ability of animals to detect and react to the spatial and non-spatial changes (Poucet 1989; Roullet et al. 1996; Thinus-Blanc et al. 1992; Usiello et al. 1998). Thus, it allows the comparison of the effects of lesions or pharmacological manipulations on two different behavioral responses (Adriani et al. 1996; Buhot et al. 1989; Poucet 1989; Thinus-Blanc et al. 1992). The present study was also aimed at determining possible differences between the two major DA receptor subclasses located in the accumbens on spatial learning. D1 and D2 receptors have been differentiated at an electrophysiological, biochemical, and behavioral level (Clark and White 1987; Missale et al. 1998; White and Wang 1986), and a localization of these receptors on different cellular populations in the striatum has been suggested (Gerfen et al 1995; Le Moine and Bloch 1995): however, to our knowledge there is only one report on a possible involvement of nucleus accumbens D1 receptors in spatial learning (Scheel-Krüger et al. 1989).

Materials and methods

Subjects

The subjects were male CD-1 outbred mice obtained from Charles River. At the time of surgery the mice were approximately 8–9 weeks old and their weights ranged from 34 to 40 g. They were housed in groups of six in 21×21×12 cm standard breeding cages placed in a room with a 12:12-h light:dark cycle (lights on 0730–1930 hours), at a constant temperature (22±1°C) with food and water freely available. The study was conducted according to Italian laws on the use of animals in experimental research and to NIH guidelines on animal care.

Novelty apparatus

The apparatus (Fig. 1) was the same used in previous studies (Poucet 1989; Roullet et al. 1996; Save et al. 1992a,b; Thinus-Blanc et al. 1992). In brief, it was a circular open field, 60 cm in diameter with a 20-cm high wall made of gray plastic material and a floor painted white and divided into sectors by black lines, placed into a soundproof room, and surrounded by a visually uniform environment, except for a striped pattern, 20 cm wide and 10 cm high (alternating 1.5-cm-wide vertical white and black bars), attached to the wall of the open field. The apparatus was illuminated by a red light (80 W) located on the ceiling, and a video camera above the field was connected to a monitor and a video recorder. Five objects were simultaneously present in the open field: a chromium-plated parallelepiped (7×4×4 cm) with irregular holes distributed on the sides and the top, a plastic cone on a transparent cylindrical base (diameter of the section 8 cm, height 6 cm), a small ladder made of gray plastic material (height 16 cm, width 5 cm, number of steps 10) inserted on a white cylindrical base (height 2 cm, diameter 7 cm), a black plastic cylinder (height 10 cm, diameter 5 cm) on a transparent Plexiglas base with a nut (height 2 cm) fixed on the top, and a white, transparent plastic spool (height 12 cm, diameter of the top and the base 5 cm). The initial arrangement was a square with a central object (plastic cone) as schematized in Fig. 1. A sixth object (named corner) was used to examine the reactivity to non-spatial change. It consisted of two gray, iron, regularly pierced squares (10×10 cm) forming a 90° angle.

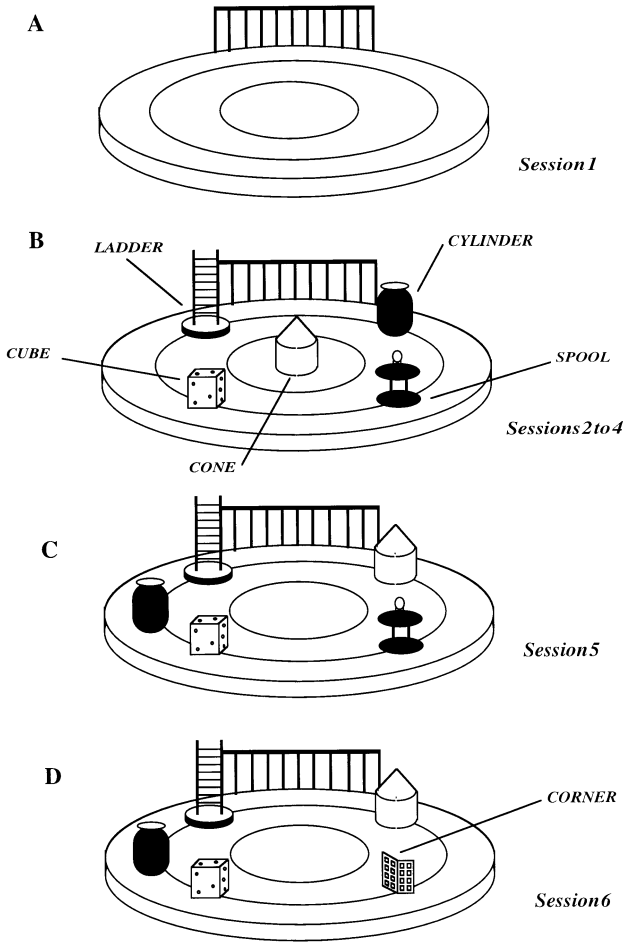

Fig. 1A–D Schematic representation of the apparatus and the object configuration over successive sessions. **A** The open field is initially empty [session 1 (S1)]. **B** In the three subsequent sessions (S2–S4) the open field is filled with objects in a particular configuration. **C** In S5 two objects are displaced (spatial novelty, DO). **D** Finally, in S6 one of the objects is substituted (non-spatial change, SO)

Surgery

Mice were anesthetized with chloral hydrate (500 mg/kg) and placed on a stereotaxic frame with mouse adapter and ear bars (Kopf Instruments). A midline incision was made, holes were drilled in the skull, and bilateral guide cannulae (7 mm in length, 0.5 mm in diameter) were implanted 2 mm above the nucleus accumbens and fixed to the skull using dental cement. The following coordinates with lambda and bregma in the same horizontal plane were used: anterior to bregma, +1.7 mm; lateral to midline ±1 mm; ventral from the dura 2 mm, according to Franklin and Paxinos (1997). Mice were then left in their home cage for a recovery period of 5–7 days.

Drugs

All drugs were diluted in saline solution, except sulpiride (D2 antagonist) which was dissolved in a minute volume of acetic acid and diluted with saline solution (NaCl 0.9%) to the final concentration, and the pH was adjusted to a value of 7.0 with NaOH. In the SCH 23390 (D1 antagonist) experiment, three doses were used (6.25 ng/0.2 µl, *n*=9; 12.5 ng/0.2 µl, *n*=8; 50 ng/0.2 µl, *n*=8). Sulpiride (D2 antagonist) was also administered in three different doses (12.5 ng/0.2 µl, *n*=9; 50 ng/0.2 µl, *n*=8; 100 ng/0.2 µl, *n*=7). In the DA experiment two doses were used (1.25 µg/side, *n*=6; 2.5 µg/side, *n*=6). Experimental groups were compared with control mice administered with the same volume of vehicle (*n*=12, *n*=12, and *n*=7, respectively, for the SCH 23390, sulpiride, and DA experiments). All mice were used only once.

Behavioral procedure

The behavioral procedure was that described by Save et al. (1992b) for the rat and Roullet et al. (1996) for mice. On the test day, mice were individually placed in a 21×21×12 cm standard plastic cage. After 20 min, they were placed into the empty open field (i.e., without objects) for a 6-min session, in order to familiarize the animal with the apparatus and to record the baseline level of locomotor activity. Subjects were then removed and placed back in the cage. An injection needle (9 mm length, 0.25 mm in diameter), connected to a 2-µl Hamilton syringe through polyethylene tubing, was placed in the guide cannula and the animals were injected with either saline solution or the drugs. The volume injected was always 0.2 µl/side, the length of the injection was 2 min/side, and the needle was left in place for an additional 30 s to allow diffusion. After a 5-min interval, animals were placed in the open field (with objects) for five successive 6-min sessions, separated by a 2-min interval, during which the subjects were returned to their cage. During sessions 2–4 (S2–S4), the objects were placed in a square configuration, with a central object (cone, object B). In S5 (spatial novelty session), the configuration was changed by displacement of two objects: the cone (B) replaced the cylinder (D), which was itself displaced at the periphery of the open field, and particularly between ladder (C) and parallelepiped (A), so that the initial square arrangement was changed to a new spatial configuration. For S6 (non-spatial novelty session), one of the familiar non-displaced objects (spool, object E) was replaced by a new object (corner, object F) in the same location (Fig. 1). All the objects were manipulated before each session.

Histological analysis

At the completion of the experiments, mice were killed by an overdose of chloral hydrate and the brain removed and fixed in formalin (4% solution). Coronal sections (60 µm) were taken and stained with cresyl violet.

Data collection and statistical analyses

Data collection was performed by a trained observer, with the use of a computer keyboard and customized software. During S2–S6, object exploration was scored as the time spent by the animal in contact with an object. A contact was defined as the subject's snout actually touching an object (for details, see Save et al. 1992a,b). *Locomotor activity* (number of sectors crossed by each animal while moving in the open field) was also scored, and a one-factor ANOVA for independent measures was performed upon these data, with Treatment as a between-subjects factor (four levels for the SCH 23390 and sulpiride: saline and three doses for each drug; three levels for DA experiment: saline and two doses for each drug). All the other data were analyzed with an analysis of variance for independent measures on the factor Treatment and repeated measures on the second factor which was Sessions for the habituation and Object Category for the spatial and non-spatial changes.

Habituation of object exploration was assessed by considering the mean duration of contacts with the five objects during each of the sessions 2, 3, and 4. A two-factor ANOVA was performed on these data, with Session (three levels: S2, S3, and S4) as a within-subject factor, and Treatment as a between-subjects factor (four and three levels, respectively, for the antagonists and the DA experiments).

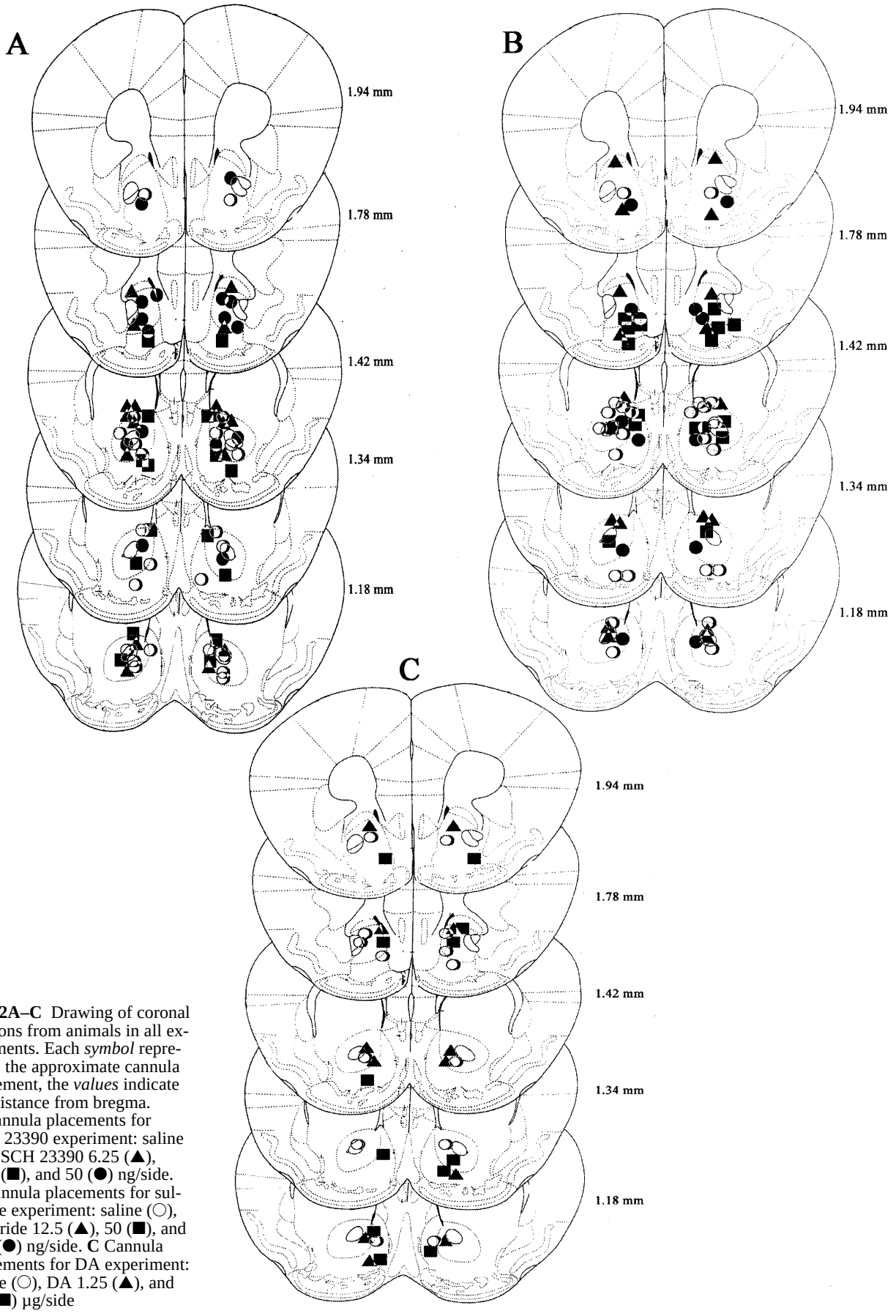

Fig. 2A–C Drawing of coronal sections from animals in all experiments. Each *symbol* represents the approximate cannula placement, the *values* indicate the distance from bregma. **A** Cannula placements for SCH 23390 experiment: saline (○), SCH 23390 6.25 (▲), 12.5 (■), and 50 (●) ng/side. **B** Cannula placements for sulpiride experiment: saline (○), sulpiride 12.5 (▲), 50 (■), and 100 (●) ng/side. **C** Cannula placements for DA experiment: saline (○), DA 1.25 (▲), and 2.5 (■) µg/side

In S5, the spatial arrangement of the objects was modified and *response to spatial change* was assessed by considering the mean time in contact with the objects belonging to each category (displaced object, DO, or non-displaced object, NDO) in S5 *minus* the mean time spent in contact with the same object category in S4. A two-factor ANOVA was performed on these data, with Object Category (two levels: DO or NDO) as a within-subject factor and Treatment as a between-subjects factor.

Finally, in S6, a non-displaced object was substituted with a new one at the same location and levels of *response to the new object* were assessed by considering the mean time in contact with the objects belonging to each category (substituted, SO, or non-substituted, NSO) in S6 *minus* the mean time spent in contact with the same object category in S5. A two-factor ANOVA was performed on these data, with Object Category (two levels: SO or NSO) as a within-subject factor and Treatment as a between-subjects factor. A simple effect analysis and/or *t*-test for paired samples were performed when appropriate.

Results

Histological verification

Figure 2 shows cannula placements for all three experiments. Only mice with correct cannula placements were included in the statistical analysis. Histological analysis shows that injection sites were located for the majority of mice in the nucleus accumbens core region. It should be mentioned, however, that no difference was observed in mice injected in the accumbens core or shell in any of the experiments performed in this study.

Effect of SCH 23390 on locomotor activity and habituation

The effects of focal administrations of D1 selective antagonist SCH 23390 on locomotor activity as mean sector crossings in all sessions are reported in Table 1. Focal administrations of the D1 antagonist did not affect the number of sectors crossed [$F(3,33)=0.5$, $P=0.65$].

Table 1 Locomotor activity (mean ± SEM) expressed as total number of crossings during the whole test (S2–S6) by mice in the different treatment groups. *Sal* Saline, *SCH* SCH 23390, *Sulp* sulpiride, *DA* dopamine

Treatment	Number of mice	Mean ± SEM
Sal	12	234.4±42.7
SCH 6.25 ng/side	9	254.2±31.6
SCH 12.5 ng/side	8	273.9±32.5
SCH 50 ng/side	8	320.6±83.4
Sal	12	287.2±35.7
Sulp 12.5 ng/side	9	209.8±47.4
Sulp 50 ng/side	8	228.0±50.9
Sulp 100 ng/side	7	76.3±12.4
Sal	7	419±106.5
DA 1.25 µg/side	6	2026±453.8*
DA 2.5 µg/side	6	2308.8±363.22*

*$P<0.05$ vs saline

The D1 antagonist, however, dose dependently decreased the time spent by the animals exploring the objects during the three habituation sessions (Fig. 3). The analysis of variance revealed a significant effect of treatment [$F(3,33)=5.25$, $P=0.0045$], a significant effect of sessions [$F(2,66)=67.9$, $P=0.0001$], but no interaction between the two effects [$F(6,66)=0.7$, $P=0.55$].

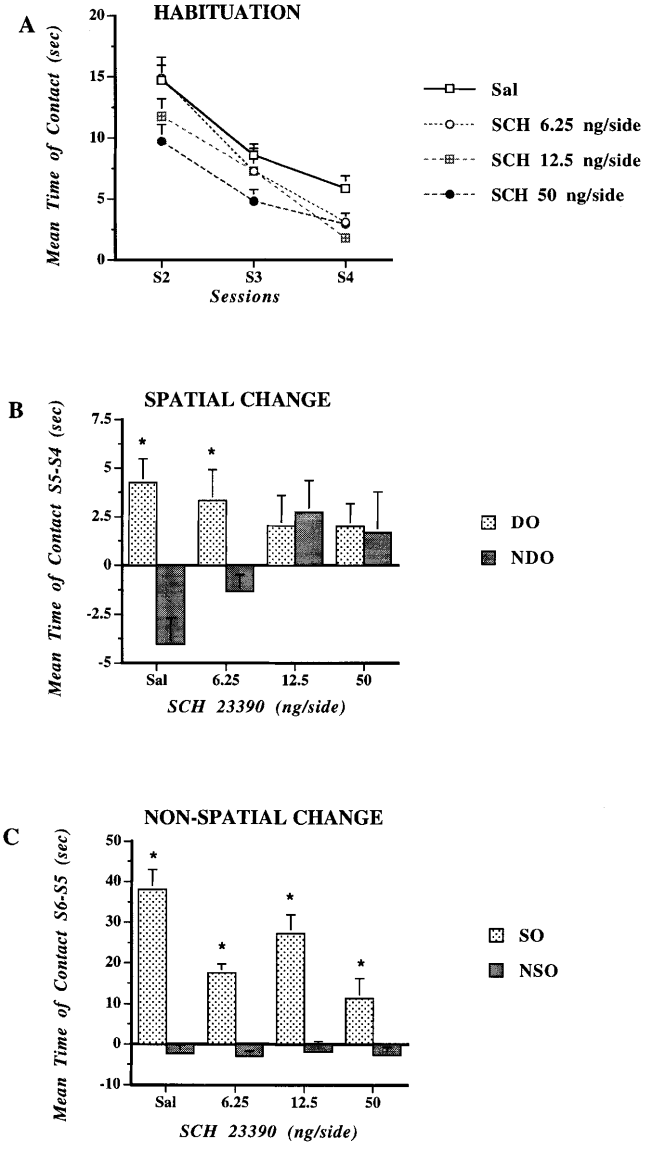

Fig. 3 Habituation (**A**) and reactivity to spatial (**B**) and non-spatial (**C**) change in mice focally administered with saline (*sal*) or SCH 23390 (*SCH*). **A** Mean duration of contacts (± SEM) with the five objects during the three habituation sessions (S2–S4). **B** The histogram represents the mean time (± SEM) spent exploring displaced objects (*DO*) or non-displaced objects (*NDO*) in S5 minus the time spent in exploring the same class of objects in the preceding session (S4). **C** The histogram represents the mean time (± SEM) spent exploring substituted (*SO*) or non-substituted objects (*NSO*) in S6 minus the time spent in exploring the same class of objects in the preceding session (S5). *Asterisks* $P<0.05$ DO vs NDO and SO vs NSO

Effect of intra-accumbens SCH 23390 on reactivity to spatial and non-spatial novelty

Figure 3 shows the effects of intra-accumbens saline and SCH 23390 on renewed exploration of DO/NDO, expressed as the difference in time spent exploring the two categories of objects in S5 and S4. Saline-administered mice re-explored the displaced objects in S5 while decreased the exploration to the non-displaced objects. Focal administrations of the D1 antagonists impaired spatial change discrimination. Drug-injected mice, in fact, do not show any difference in the re-exploration of the two object categories (DO and NDO), due to an increased re-exploration of non-displaced objects. The analysis of variance revealed a significant main object category effect (DO/NDO) [$F(1,33)=28.06$, $P=0.0001$] and a significant interaction between the two effects [$F(3,33)=13.39$, $P=0.0001$], but no significant treatment effect [$F(3,33)=0.597$, $P=0.6$]. The *post hoc* comparison revealed a significant difference between object category only for saline and the low dose of SCH 23390 (Fig. 3).

Figure 3 shows the effects of saline or SCH 23390 administration on reactivity to non-spatial novelty expressed as the difference in time spent exploring the two categories of objects in S6 and S5. Saline-injected mice reacted to the novel object by increasing exploration to it. Focal administration of the D1 antagonist reduced the time spent by the animals in contact with the new object. Analysis of variance revealed a significant effect of treatment [$F(3,33)=6.35$, $P=0.0016$], a significant object effect [$F(1,33)=114.47$, $P=0.0001$], and a significant interaction between the two effects [$F(3,33)=6.14$, $P=0.0019$]. *Post hoc* comparisons revealed a significant difference between object category for saline as well as the three groups of SCH 23390-treated mice (Fig. 3).

Effect of intra-accumbens sulpiride on locomotor activity and habituation

Table 1 reports the effects of sulpiride on locomotor activity, expressed as sector crossings, in S2–S6. Focal administration of the D2 selective antagonist induced a decrease of the number of sectors crossed and the one-factor ANOVA revealed a significant effect of treatment [$F(3,32)=4.41$, $P=0.0105$].

Figure 4 shows habituation of the saline- and sulpiride-injected groups. Focal administration of the D2 receptor antagonist decreased the time spent by the mice exploring the objects during habituation. The analysis of variance (ANOVA) revealed a significant effect of treatment [$F(3,32)=7.96$, $P=0.0004$], a significant effect of sessions [$F(2,32)=50.59$, $P=0.0001$], and a significant interaction between treatment and sessions [$F(6,32)=3.78$, $P=0.0027$].

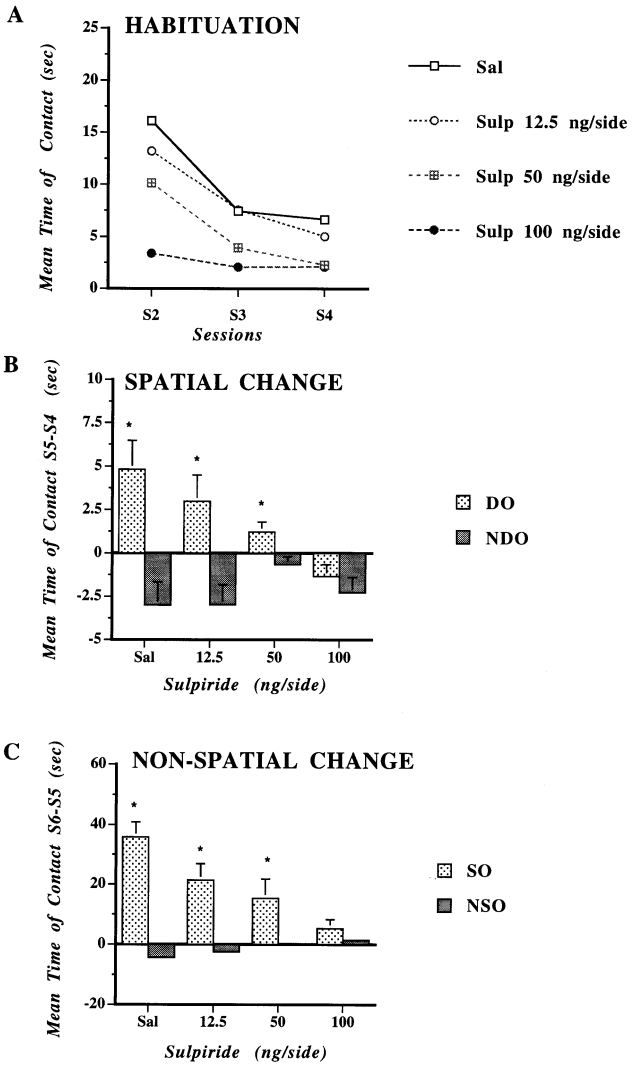

Fig. 4 Habituation (**A**) and reactivity to spatial (**B**) and non-spatial (**C**) change in mice focally administered with saline (*sal*) or sulpiride (*sulp*). **A** Mean duration of contacts (± SEM) with the five objects during the three habituation sessions (S2–S4). **B** The histogram represents the mean time (± SEM) spent exploring displaced (*DO*) or non-displaced objects (*NDO*) in S5 minus the time spent in exploring the same class of objects in the preceding session (S4). **C** The histogram represents the mean time (± SEM) spent exploring substituted (*SO*) or the non-substituted objects (*NSO*) in S6 minus the time spent in exploring the same class of objects in the preceding session (S5). *Asterisks* $P<0.05$ DO vs NDO and SO vs NSO

Effect of intra-accumbens sulpiride on reactivity to spatial and non-spatial novelty

Figure 4 shows the effect of focal administration of sulpiride on exploration of spatial and non-spatial novelty (DO/NDO), expressed as the difference in time spent in contact with the two categories of objects in S5 minus S4. Saline-injected mice selectively renewed exploration of DO category. Focal administration of the D2 antagonist dose dependently reduced DO without affecting NDO renewal of exploration (Fig. 4). The analysis of

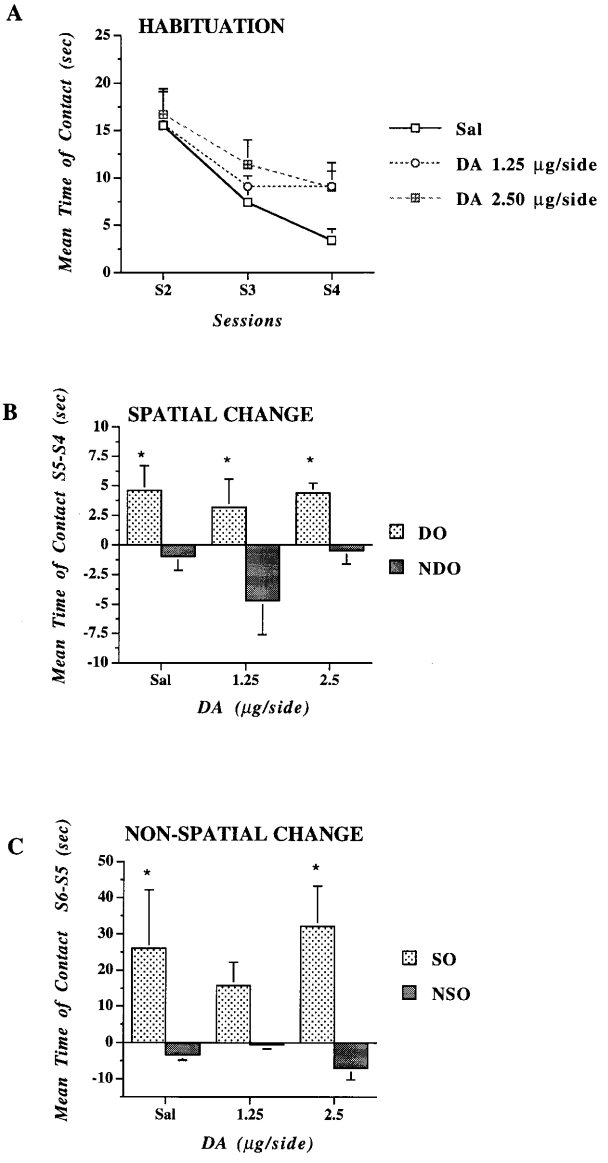

Fig. 5 Habituation (**A**) and reactivity to spatial (**B**) and non-spatial (**C**) change in mice focally administered with saline (*sal*)or dopamine (*DA*). **A** Mean duration of contacts (± SEM) with the five objects during the three habituation sessions (S2–S4). **B** The histogram represents the mean time (± SEM) spent exploring displaced (*DO*) or non-displaced objects (*NDO*) in S5 minus the time spent in exploring the same class of objects in the preceding session (S4). **C** The histogram represents the mean time (± SEM) spent exploring substituted (*SO*) or non-substituted objects (*NSO*) in S6 minus the time spent in exploring the same class of objects in the preceding session (S5). *Asterisks* $P<0.05$ DO vs NDO and SO vs NSO

variance revealed a significant main object category effect (DO/NDO) [$F(1,32)=17.699$, $P=0.0002$], a significant interaction between two factors [$F(3,32)=2.894$, $P=0.0504$], but no significant treatment effect [$F(3,32)=1.804$, $P=0.1662$].

The effects of intra-accumbens saline and sulpiride on reactivity to non-spatial novelty are shown in Fig. 4. Sulpiride also in this case dose dependently reduced SO renewal of exploration. The ANOVA revealed a significant main effect of object category (SO/NSO), [$F(1,32)=54.945$, $P=0.0001$], a significant interaction between two factors [$F(3,32)=7.896$, $P=0.0004$], but no significant treatment effect [$F(3,32)=4.068$, $P=0.0148$].

Effect of intra-accumbens DA on locomotor activity and habituation

DA induced a dose-dependent increase in locomotor activity (Table 1). The ANOVA on the total number of sector crossings in all sessions revealed a significant treatment effect [$F(2,16)=10.3$, $P=0.0013$]. The hyperactivity effect induced by DA was still evident in S6, the mean effect ± SEM in S6 being: 48.8±17.9, 348.3±96, and 407±95.5, respectively, for saline-, DA 1.25-, and DA 2.5 µg/side-injected groups.

Figure 5 shows the effect of focal administration of saline and the doses of DA on habituation in the first three sessions. The mean time spent by the mice exploring the overall set of objects decreases over sessions in the saline- and the drug-treated groups. DA administration induced a slight increase in exploration which did not reach significance. Analysis of variance revealed a significant main sessions effect [$F(2,32)=14.175$, $P=0.0001$], but no treatment effect [$F(2,216)=1.57$, $P=0.24$] or interaction between the two main effects [$F(4,32)=0.58$, $P=0.67$].

Effect of intra-accumbens DA on reactivity to spatial and non-spatial novelty

Figure 5 shows the effect of focal administrations of saline and the two doses of DA on reactivity to the spatial change expressed as the difference in time spent exploring the two categories of objects in S4 and S5. DA did not induce any significant effect on reactivity to spatial change; the analysis of variance revealed only a significant effect of object category [$F(1,16)=35.68$, $P=0.0001$], but no effect of treatment [$F(2,16)=0.79$, $P=0.47$] or interaction between the two main effects [$F(2,16)=0.74$, $P=0.48$]. Simple effects analysis revealed a significant difference in reactivity to DO and NDO for all three experimental groups.

Figure 5 shows the effects of intra-accumbens saline or DA on reactivity to a novel object. All three groups reacted to the new object and DA administration did not affect the response in any way. Analysis of variance revealed only a significant effect of object category (SO/NSO) [$F(1,16)=14.89$, $P=0.0014$], but no treatment effect [$F(2,16)=0.151$, $P=0.86$] or interaction between the two factors [$F(2,16)=0.77$, $P=0.48$]. Simple effect analysis revealed a significant difference for object category for the groups injected with saline and the high dose, but not the low dose, of DA.

Discussion

In the present study we report on the effects of manipulating DA receptors in the nucleus accumbens in a non-associative task designed to determine the ability of mice to discriminate discrete spatial and non-spatial changes (Poucet 1989; Roullet et al. 1996; Thinus-Blanc et al. 1996). In all three experiments of this study, saline-infused control mice showed a similar decreased tendency to explore the set of objects over the first three sessions (S2–S4), suggesting that they were habituating to the objects as well to the position of the objects in the open field. When in S5, two of the objects were displaced in the open field, i.e., the spatial relationship among them was changed, control mice increased their exploration of the displaced objects in comparison to the preceding session. Finally, when a new object was placed in the arena in S6, increased exploration directed toward the new object was observed. The renewed exploration toward the displaced objects in S5 indicates a selective pattern of response toward the change which had occurred in the environment, suggesting that the animals perceive the spatial change in the environment and that the new arrangement is compared to the internal representation of the original situation. The explorative reactivity to the non-spatial change in S6 represents a mean to assess the ability to detect a change in the environment (Poucet 1989). It should be mentioned, however, that the emotional impact of the novel object in S6 is much higher than that induced by the rearrangement of the familiar objects in S5. It has indeed been suggested that reactivity to spatial and non-spatial changes might be partially subserved by separate mechanisms (Adriani et al. 1996, 2000; Sargolini et al. 1999).

In this, as well as in all focal injection studies, a substantial issue is the possible drug diffusion beyond the injected structure. However, we do not think that the effects observed in the present study are due to action of the drugs outside the accumbens. For instance, the ventricles are very near the nucleus accumbens, but a possible spreading into the ventricles would have been expected to induce effects similar to those induced by systemic administrations of the same drugs (Adriani et al. 2000) and this was not the case. Nevertheless, drug spreading into proximal regions cannot be completely ruled out and should be taken into consideration.

In the first experiment we investigated the effects of intra-accumbens D1 antagonist SCH 23390. Blockade of D1 receptors in this structure did not affect locomotor activity. SCH 23390 administration was also ineffective in altering the cause of habituation at any dose. In all groups a similar decrease in the time spent in the exploration of the all set of objects was observed throughout S2–S4. It should be noticed, however, that SCH 23390 decreased object exploration in S2 as well as in the subsequent sessions. In S5, SCH 23390 impaired reactivity to spatial change. D1 antagonist-infused groups, in fact, re-explored in a similar manner both displaced and non-displaced objects. It is interesting to note that the lack of specific re-exploration directed toward the displaced objects in drug-treated groups is due to renewed exploration of the NDO category in S5. Finally, in S6 all SCH 23390-treated groups showed increased exploration toward the new object, but not the familiar one. Drug treatment, however, reduced in S6 the impact of novelty, as evidenced by the shorter time spent in object exploration in comparison to saline-injected mice. It should be mentioned that SCH 23390 has been demonstrated to act also as a 5-HT2 antagonist, therefore, it cannot be completely excluded that the effects observed are also mediated by an action at these receptors. It should be considered, however, that systemic administration of 5-HT2 agonists rather than antagonists has been shown to impair spatial learning (Kant et al. 1998).

Focal administration of SCH 23390 decreased exploration of objects during the first three sessions but this effect was not associated with reduced locomotor activity. This indicates a clear dissociation between the effects of the D1 antagonist on locomotor activity and exploration. Reduced gathering of information during the first three sessions could explain the lack of selective reactivity to DO in S5. However, in our opinion, it is difficult to ascribe the response observed in S5 to the reduced exploration of the objects during habituation. In this case a lack of discrimination of the non-spatial change should have also been expected, whereas all SCH 23390-administered groups explored the novel object more than the familiar objects in S6. The generalized response directed toward both object categories in S5 could indicate that the animals perceive a change but are not able to respond to it appropriately (Sargolini et al. 1999). The slight decrease found in SO reactivity after intra-accumbens SCH 23390 focal administration is consistent with the reduction in object exploration during habituation. This observation further supports previous reports of a reduced exploration induced by the D1 antagonist (Stahle and Ungerstedt 1986).

In contrast to SCH 233390, intra-accumbens administration of the D2 antagonist, sulpiride, induced a similar, dose-dependent decrease on all variables measured. Focal administration of the drug decreased both locomotor activity and the time spent in contact with all the objects during S2. The habituation pattern of the mice injected with low doses of sulpiride did not differ from that of saline-injected animals, only the high dose injected group did not habituate to the objects. In our opinion, it is difficult to ascribe this effect to a specific action of sulpiride on the habituation process. Rather it could be due to the low level of exploration shown by the animals during S2. Blockade of D2 receptors in the nucleus accumbens induced a dose-dependent decrease in reactivity to both changes, DO and SO, respectively, in S5 and S6. This effect was expressed as a dose-dependent decrease in the renewal of exploration of DO and SO categories, with no effect on the exploration of non-displaced or familiar objects in the two sessions.

Finally, intra-accumbens DA increased locomotor activity, thus confirming previous studies demonstrating a

stimulatory role of the mesoaccumbens dopaminergic system on locomotion (Kelly et al. 1975; Mele et al. 1998). On the contrary, no significant difference was observed between saline- and DA-injected groups, on either exploration of the overall set of objects in S2 or the habituation pattern. Focal administration of DA was also ineffective on the reactivity to spatial change in S5. It is interesting to note that in S6 no effect of DA focal administration was observed on reactivity to novelty. Focal administrations of DA would have been expected to exert opposite effects in comparison to that induced by selective antagonists. The behavioral response observed in this study is not completely consistent, since DA increased locomotor activity, but did not affect in a significant way any of the other parameters measured. This could possibly be due to a ceiling effect in the exploration of the objects shown by control mice (see Adriani et al. 2000).

On the basis of the effects observed after systemic administrations of DA agonists and antagonists in the same task, we have recently suggested a facilitatory role of DA transmission on spatial learning and an inhibitory role on novelty exploration. The inhibitory role on novelty exploration was suggested on the basis of decreased SO exploration observed after systemic administrations of both direct and indirect DA agonists (Adriani et al. 2000), and it is supported by other evidence (Misslin and Ropartz 1981a). In this study, focal administration of DA into the nucleus accumbens, did not confirm this observation: on the contrary an inhibitory effect was observed after focal administrations of DA antagonists on SO exploration in S6, thus suggesting that the inhibitory effect of enhanced DA transmission on novelty exploration is mediated by different neural systems (Adriani et al. 2000). There are several lines of converging evidence suggesting an involvement of ventral tegmental area–amygdala projections in conditioned fear response (Killcross et al. 1997; Nader and Le Doux 1999). It is therefore conceivable that the effect observed after systemic administrations of DA agonists could be due to enhanced DA transmission in this area.

Striatal DA depletion has been demonstrated to impair spatial navigation in the Morris water maze, though it was suggested that the impairment could not be attributed to impaired spatial learning, but rather to more general "regulatory impairments" (Hagan et al. 1983; Winn and Robbins 1985). The effects observed after D2 receptor blockade in the accumbens is consistent with this hypothesis. In fact, the reduction observed after sulpiride administration on all the behavioral parameters measured suggests the involvement of this receptor population in a general process common to all the responses. It is worth noting that a similar pattern of response was observed in the same task after haloperidol systemic administrations (Roullet et al. 1996).

On the other hand, SCH 23390 seems to be more selective for spatial learning. Focal administration of SCH 23390 impaired, like sulpiride, the exploration of the object in the first three sessions and selective re-exploration of displaced objects in S5. However, no effect was observed after D1 antagonist administration on locomotor activity and reaction to novelty was only partially impaired. It is also difficult to postulate that SCH 23390 was acting on a common mechanism to modulate DO and SO responses. In fact, sulpiride reduces DO reactivity decreasing re-exploration directed toward the displaced objects and a similar decrease is observed after D2 receptor blockade on re-exploration of SO in S6. On the contrary, SCH 23390-induced impairment in discrimination of spatial change is due to the renewed exploration of non-displaced objects and no decrease was observed on DO reactivity, while the effect on novelty reaction was due to a decrease in the exploration of SO category. If the D1 antagonist was acting on the same process to modulate reactivity to DO and SO, a similar effect should have been expected. Therefore, it is possible to suggest that SCH 23390 is acting independently on two different processes: the intensity of response to novel objects, and the ability to encode/transmit spatial information.

Overall this study demonstrates a facilitatory effect of increased DA transmission in the nucleus accumbens on the ability to react to both spatial and non-spatial changes. On the basis of the effects induced by the D1 receptor antagonist, we suggest that these two responses depend on different functional mechanisms within this structure, and that D1 receptors seem more selective than D2 receptors for the encoding/transmission of spatial information. In this regard it should be noted that D1 receptors have been demonstrated to be involved in working memory processes in other brain structures (Muller et al. 1998; Sawaguchi and Goldman-Rakic 1991; Seamans et al. 1998) and that a role of striatum in spatial working memory has been recently suggested in humans (Postle and D'Esposito 1999). Nucleus accumbens receives afferent projections from amygdala, hippocampus, and prefrontal cortex which have been demonstrated to modulate separate aspects of novelty processing (Poucet 1989; Sargolini et al. 1999; Save et al. 1992a). Considering the neuroanatomical position of the accumbens and its role in mediating motor output it seems conceivable that these different contextual items of information are relayed in this structure in order to promote/potentiate new behavioral responses (Pennartz et al. 1994).

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