

Dopamine–Glutamate Interplay in the Ventral Striatum Modulates Spatial Learning in a Receptor Subtype-Dependent Manner

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The ventral striatum (VS) is characterized by a distinctive neural architecture in which multiple corticolimbic glutamatergic (GLUergic) and mesolimbic dopaminergic (DAergic) afferents converge on the same output cell type (the medium-sized spiny neuron, MSN). However, despite the gateway function attributed to VS and its involvement in action selection and spatial navigation, as well as the evidence of physical and functional receptor–receptor interaction between different members of ionotropic GLUergic and DAergic receptors, there is no available knowledge that such reciprocal interaction may be critical in shaping the ability to learn novel spatial and non-spatial arrangement of stimuli. In this study, it was evaluated whether intra-VS bilateral infusion of either *N*-methyl-D-aspartate (NMDA) or α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-selective antagonists may suppress the ability to detect spatial or non-spatial novelty in a non-associative behavioral task. In a second set of experiments, we further examined the hypothesis that VS-mediated spatial information processing may be subserved by some preferential receptor–receptor interactions among specific GLUergic and DAergic receptor subtypes. This was assessed by concomitant intra-VS infusion of the combination between subthreshold doses of either NMDA or AMPA receptor antagonists with individual D1 or D2 receptor blockade. The results of this study highlighted the fact that NMDA or AMPA receptors are differentially involved in processing of spatial and non-spatial novelty, and showed for the first time that preferential NMDA/D1 and AMPA/D2 receptor–receptor functional communication, but not NMDA/D2 and AMPA/D1, is required for enabling learning of novel spatial information in the VS.

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INTRODUCTION

The ventral striatum (VS) is characterized by the convergence of dopamine (DA) terminals, and thalamic and corticolimbic (prefrontal cortex, hippocampus, amygdala) glutamate (GLU) signals onto the same intrinsic GABAergic medium-sized spiny neurons (MSNs). In particular, mid-brain DA terminals form symmetric synaptic contacts with dendritic shafts while glutamatergic (GLUergic) axons establish (asymmetric) synaptic contacts on the heads of the same MSN spines (Bouyer *et al*, 1984; Freund *et al*, 1984; Smith *et al*, 1994; Sesack *et al*, 2003). This morphological arrangement offers the structural basis and the architectural framework for an interaction between dopaminergic

(DAergic) and GLUergic inputs. Indeed, a large body of neurochemical and electrophysiological studies supports a functional interaction between these two systems in the modulation of GABAergic MSNs in the striatum (Cepeda and Levine, 1998; West *et al*, 2003; Surmeier *et al*, 2007). In particular, the hypothesis of functional receptor–receptor interaction has been corroborated by a series of elegant studies showing that the modulatory action exerted by DAergic receptors on ionotropic GLUergic signals in neostriatal neurons depends on the subtypes of receptors involved (Levine *et al*, 1996; Cepeda and Levine, 1998). Activation of D1 receptors enhances *N*-methyl-D-aspartate (NMDA)-mediated whole-cell currents in neostriatal slices whereas stimulation of D2 receptors produces either negligible effects or inhibitory action on the responses evoked by non-NMDA receptors (Cepeda *et al*, 1993; Cepeda *et al*, 1998; Flores-Hernandez *et al*, 2002).

From a behavioral point of view, the VS has been traditionally linked to motivational processes and addiction (Ikemoto and Panksepp, 1999; Nicola *et al*, 2000; Kelley, 2004; Carlezon and Thomas, 2009; Russo *et al*, 2010).

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Nevertheless, the vast array of signals conveyed from corticolimbic regions has suggested a role of this structure also in the acquisition and storage of information essential to code spatial information and novel environmental configuration (Setlow, 1997; Tabuchi *et al.*, 2000). This hypothesis is sustained by behavioral evidence demonstrating that temporary or permanent manipulations of the VS impair performance in spatial learning task (Coccarello *et al.*, 2000; Rouillet *et al.*, 2001). As regards the receptor system involved, robust pharmacological evidence demonstrates that both DA and GLU receptors are crucial in processing space representation (Ploeger *et al.*, 1994; Maldonado-Irizarry and Kelley, 1995; Coccarello *et al.*, 2000; Rouillet *et al.*, 2001; Sargolini *et al.*, 2003a). However, despite the evidence demonstrating the existence of the neurobiological substrate for a physical interaction between DA and GLU receptors very little is known about a possible functional interaction between these two receptor systems in the modulation of the behavioral output of the VS. In fact, with few exceptions (Smith-Roe and Kelley, 2000; Di Ciano *et al.*, 2001; David *et al.*, 2005; Ferretti *et al.*, 2005; Hernandez *et al.*, 2005), the role of NMDA and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-type glutamate receptors (NMDA-R and AMPA-R, respectively) or DA D1 and D2 receptors in the VS (relatively to learning and memory processes) has always been considered separately (Maldonado-Irizarry and Kelley, 1995; Usiello *et al.*, 1998; Smith-Roe *et al.*, 1999; Coccarello *et al.*, 2000; Sargolini *et al.*, 2003a; Sargolini *et al.*, 2003b; Mele *et al.*, 2004).

Moving from this consideration, in the first set of experiments, we evaluated whether intra-VS selective blockade of either NMDA or AMPA receptors may impair the ability of mice to encode novel spatial arrangement information. In the second set of experiments we tested the hypothesis that selective receptor-receptor interactions (eg, D1-NMDA, D2-NMDA, D1-AMPA or D2-AMPA) could mediate spatial learning in the VS. For this purpose, we combined the doses of 2-amino-5-phosphonopentanoic acid (AP-5) and 6,7-Dinitroquinoxaline-2,3(1H,4H)-dione (DNQX) found to be ineffective in the first set of experiments with doses of the D1 (SCH23390) or the D2 (sulpiride) DA receptor antagonists known to be ineffective in the same spatial learning task (Coccarello *et al.*, 2000).

MATERIALS AND METHODS

Subjects

Male CD-1 outbred mice were obtained from Charles River (Como, Italy). At the time of surgery subjects were ~8–9-week-old and their weights ranged from 33 to 40 g. Mice 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 h), at constant temperature (22 ± 1 °C) with food and water freely available. All the experiments were performed according to the Italian laws on the use of animals in experimental research and NIH guidelines on animal care.

Surgery

Mice were anaesthetized with chloral hydrate (500 mg/kg) and placed on a stereotaxic frame with mouse adapter and

ear bars (David Kopf Instruments, Tujunga, California). 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 VS and fixed 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 purchased from Sigma-Aldrich, Italy. AP-5 was used as selective and competitive NMDA antagonist. For the experiment of intra-VS AP-5 infusion four doses were infused, as follows: 3.125, 6.25, 25, and 50 ng/side. DNQX was used as competitive AMPA glutamate receptor antagonist. DNQX was infused at the following doses: 0.25, 0.5, and 1 ng/side. (–)Sulpiride (SULP) and R(+)-SCH-23390 hydrochloride (SCH) were chosen as selective antagonists at D2 and D1 receptor, respectively. The doses of D1 (6.25 ng/side) and D2 (6.25 ng/side) antagonists used were determined on the basis of previous results (Coccarello *et al.*, 2000). Except for SULP and DNQX, all drugs were diluted in 0.9% NaCl saline solution. SULP was dissolved in a drop of 0.1 M glacial acetic acid and then diluted with saline solution (NaCl 0.9%) to the final concentration, with the pH adjusted to 7.0 with NaOH. DNQX was dissolved in a minute volume of 50% dimethylsulfoxid (DMSO)/50% saline, then the volume adjusted in a solution of 2% DMSO. In the experiment of interaction between NMDA and DA receptors (NMDA/DA) different drugs were infused, either alone or in combination, at the following doses: AP-5 3.125 ng/side; SCH 6.25 ng/side, and SULP 6.25 ng/side. In the experiment of interaction between AMPA and DA receptors (AMPA/DA) different drugs were infused, either alone or in combination, at the following doses: DNQX 0.25 ng/side; SCH 6.25 ng/side, SULP 6.25 ng/side, and DNQX 0.25 ng/side. Each experimental group was compared with intra-VS vehicle-infused control mice ($n = 8$, $n = 8$, $n = 9$, $n = 9$, respectively, for the AP-5, DNQX, NMDA/DA, and AMPA/DA experiments). All agents were infused in a volume of 0.2 µl/side and all mice were used only once.

Apparatus

The apparatus (Figure 1) used for the study was the same as in previous reports (Rouillet *et al.*, 2001). It consists in a circular open field, 60 cm in diameter with a 20-cm high wall made of gray plastic material and a white-painted floor divided into sectors by black lines. The arena was placed into a soundproof room surrounded by a visually uniform environment, except for a striped pattern (20 cm wide and 10 cm high), attached to the wall of the open field. The apparatus was illuminated by a red light (80 W) and a video camera above the field was plugged to a monitor and a video recorder. Five objects were simultaneously present in the open field: a chromium-plated parallelepiped (7 × 4 × 4 cm); a plastic cone on a transparent cylindrical base (height 6 cm, diameter 8 cm); a small ladder-like item made of gray plastic material (height 16 cm, width 5 cm,

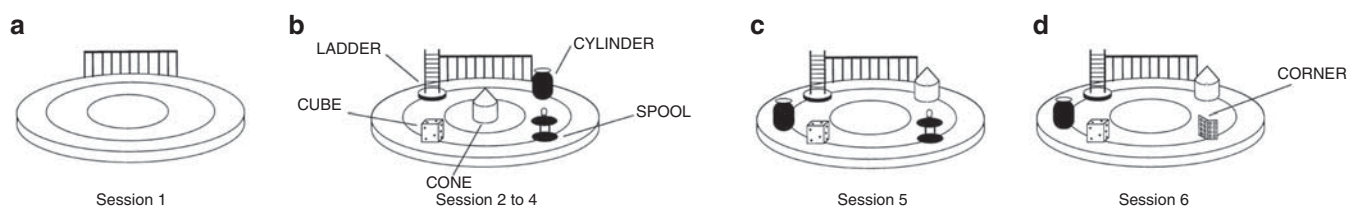


Figure 1 Schematic representation of the experimental circular arena and object arrangement over successive six sessions. (a) The empty open field (S1); (b) object spatial configuration during habituation sessions (S2–S4); (c) object spatial displacement during the spatial novelty session (S5); (d) object substitution during the non-spatial novelty session (S6).

number of steps 10) inserted on a cylindrical base (height 2 cm, diameter 7 cm); a 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 transparent plastic spool (height 12 cm, diameter of the top, and the base 5 cm). The initial arrangement was square-like with a central object (plastic cone), as schematized (Figure 1). A sixth object (named corner) was used to assess the reactivity to non-spatial novelty. It consisted of two gray regularly pierced iron squares (10 × 10 cm) forming a 90° angle.

Assessment of Reactivity to Spatial and Non-spatial Novelty and Intra-VS Infusion Procedure

The ability to react to spatial and non-spatial novelty was investigated accordingly to a previously described procedure (Coccarello *et al.*, 2000) (Figure 1). On the test day, mice were individually placed in a 21 × 21 × 12 cm standard plastic cage. After 20 min, mice were placed into the empty open field (ie, without objects) for a 6-min session in order to get acquainted with the experimental apparatus and to record the baseline level of locomotor activity (Figure 1a). 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 vehicle solution, single drugs or the combination between different agents. The duration of the infusion 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 back in the open field (with objects) for five successive 6-min sessions, separated by a 2-min interval, during which mice were returned to their cage. During sessions 2–4 (S2–S4), the objects were placed in a square-like configuration, with a central object (cone, object B) (Figure 1b). In S5 (spatial novelty session), the configuration was changed by displacing two objects: the cone (B) replaced the cylinder (D), which was itself displaced at the periphery of the open field (between ladder (C) and parallelepiped (A)), so that the initial square arrangement was changed to a new spatial arrangement (Figure 1c). In S6 (non-spatial novelty session), one of the familiar non-displaced objects (NDOs; spool, object E) was replaced by a new object (corner, object F) in the same location (Figure 1). All the objects were touched and manipulated before each session.

Histological Analysis

At the end of each experiment, mice were killed by an overdose of chloral hydrate, the brain removed and then

fixed in formaldehyde (4% solution). Cannulae placements were verified by inspection of cryostat-collected serials 60 μm coronal sections of VS, stained with cresyl violet. A schematic drawing of coronal sections from Franklin and Paxinos (1997) all VS-infused animals is depicted in Figure 2.

Data Collection and Statistical Analyses

Data collection was carried out by a trained observer blind to treatment via 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 (Roulet *et al.*, 2001). Locomotor activity (number of sectors crossed by each animal while moving in the open field) was also scored. One-way ANOVA was carried out on these data for experiments 1 and 2, with treatment (NMDA or AMPA, respectively) as between-subjects factor. A two-way ANOVA was used for experiment 3 and 4, with either NMDA and DA or AMPA and DA treatments as between-group factors. Habituation to objects was assessed by considering the mean duration of contacts with the five objects during sessions 2, 3, and 4. One-way repeated measures ANOVA was carried out, with either NMDA or AMPA as between-subjects factor (experiments 1 and 2, respectively) and Sessions (three levels: S2, S3, and S4) as within-subject factor. A two-way repeated measures ANOVA, with either NMDA and DA or AMPA and DA treatments as between-group factors and sessions (three levels: S2, S3, and S4) as within-subject factor was used for the experiments (3 and 4, respectively) of receptor interaction. In S5, the spatial arrangement of objects was modified and reactivity to spatial novelty was assessed by considering the mean time of contact with the objects belonging to each category (displaced object, DO and NDO) in S5 minus the mean time spent in contact with the same object category in S4. One-way repeated measures ANOVA was carried out with either NMDA or AMPA as between-subjects factor (experiments 1 and 2, respectively) and spatial novelty (two levels: DO, NDO) as repeated measures. Data for spatial change detection of experiment 3 and 4 were analyzed using two-way repeated measures ANOVA, with either NMDA and DA or AMPA and DA treatments as between-group factors and spatial novelty (two levels: DO, NDO) as repeated measure. Finally, in the last session (S6), a non-previously DO was substituted with a new one at the same location (non-spatial novelty), and levels of reactivity to the novel 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

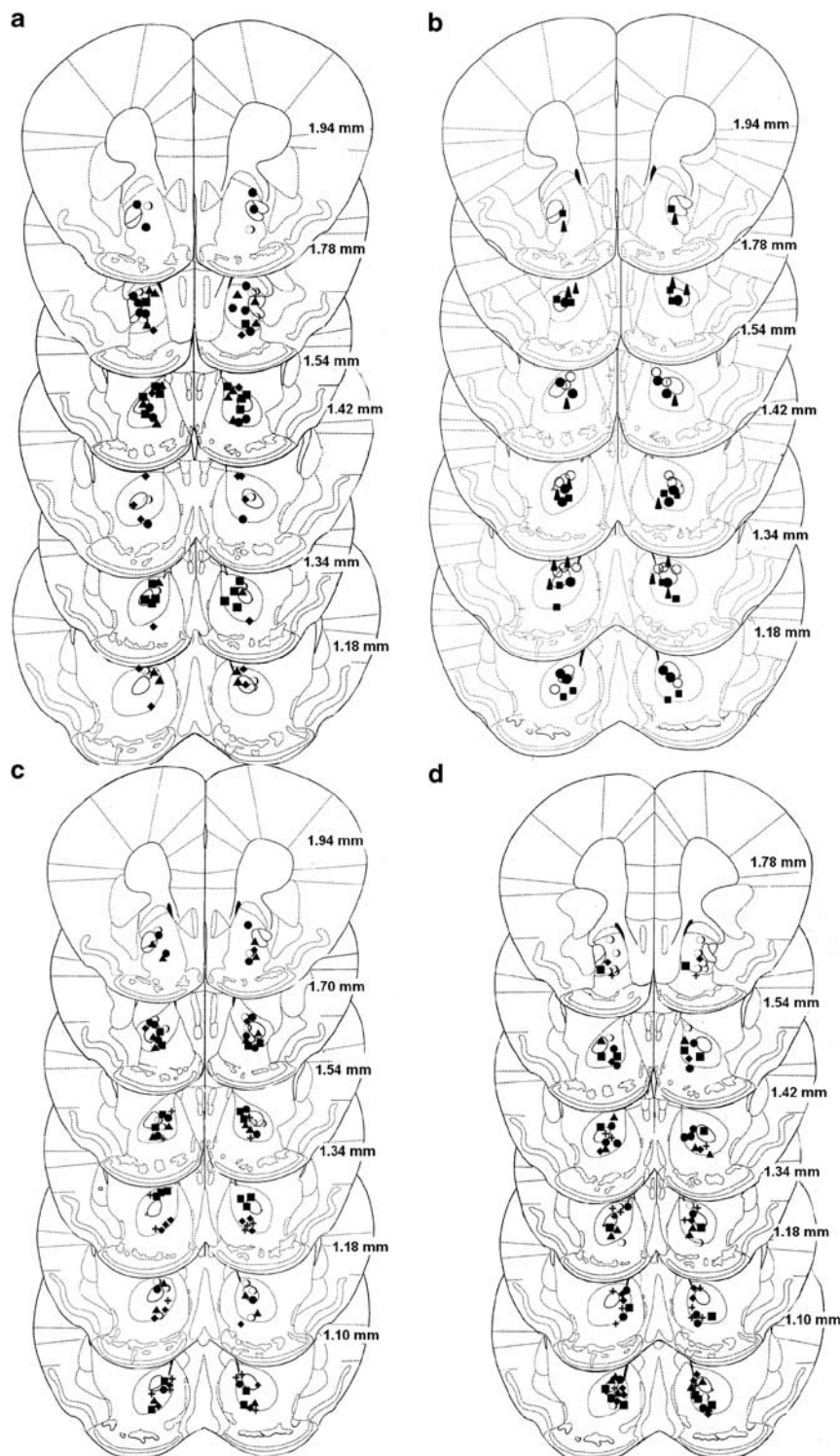


Figure 2 Schematic drawing of coronal sections from all ventral striatum (VS)-infused animals. Each symbol represents the approximate cannula placement, and the values specify the anteroposterior coordinate in relation to bregma. (a) Experiment 1: vehicle (open circle), AP-5 3.125 (filled upright triangle), AP-5 6.25 (filled square), AP-5 25 (filled circle), AP-5 50 (filled rhomb). (b) Experiment 2: vehicle (open circle), DNQX 0.25 (filled upright triangle), DNQX 0.5 (filled square), DNQX 1 (filled circle). (c) Experiment 3: vehicle (open circle), SCH 6.25 (filled upright triangle), Sulp 6.25 (filled square), AP-5 6.25 (filled circle), SCH + AP-5 (filled rhomb), Sulp + AP-5 (cross). (d) Experiment 4: vehicle (open circle), SCH 6.25 (filled upright triangle), Sulp 6.25 (filled square), DNQX 0.25 (filled circle), SCH + DNQX (filled rhomb), Sulp + DNQX (cross). All dose are expressed as ng/site. The anatomical drawings were modified from Franklin and Paxinos (1997).

mean time spent in contact with the same object category in S5. The level of re-exploration for each object category in S6 was analyzed using a one-way repeated measures ANOVA

in experiment 1 and 2 (with NMDA and AMPA as between group factor), and two-way repeated measures ANOVA in experiments 3 and 4 (with either NMDA and DA or

AMPA and DA treatments as between-group factors), and non-spatial novelty (two levels: SO or NSO) as repeated measure. Differences among groups were considered significant when $P \leq 0.05$. Tukey HSD test was used to carry out *post hoc* analyses.

RESULTS

Histological Verification

Figure 2 is a diagrammatic representation of atlas coronal sections illustrating cannulae placement for dose-response experiments (AP-5 and DNQX infusions) as well as for the experiments of NMDA/DA and AMPA/DA receptor interaction. Only mice with correct cannula placement were included in the statistical analysis. Histological verification shows that sites of drug infusion were uniformly distributed throughout groups in the different experiments. Histological analysis shows that the injection site is located in the VS core for the majority of mice.

Experiment 1: Intra-VS AP-5 Infusions

Locomotor activity and habituation. As shown in Table 1 bilateral intra-VS infusion of the NMDA competitive antagonist, AP-5, did not affect locomotor activity (mean number of sectors crossed) at any of the doses used. The ANOVA did not reveal significant any effect of the treatment. Moreover, the infusion of AP-5 did not alter the exploratory activity expressed by the animals on the whole set of the objects during the three sessions of habituation (Table 1). The ANOVA revealed only a significant effect of sessions ($F_{2,76} = 95.006$, $P < 0.001$).

Reactivity to spatial and non-spatial novelty. Figure 3 shows the effects of bilateral intra-VS vehicle and AP-5 injections on the renewal of exploration of DO/NDO, expressed as difference in the time spent exploring the two object categories in S5 and S4. Control mice re-explored the DOs in S5 (DO) while decreased the exploration towards NDOs. Intra-VS blockade of NMDA receptors induced a

Table 1 Entire Set of Drug Infusion Experiments

Intra-VS drug Infusion	Mice	Activity	Habituation		
		S2-S6	S2	S3	S4
Experiment 1:					
Vehicle	8	291.62 ± 23.54	14.92 ± 2.03	6.85 ± 0.88	3.39 ± 0.61
AP-5 3.125 ng	9	234.44 ± 21.15	11.57 ± 2.08	4.88 ± 0.68	2.93 ± 0.78
AP-5 6.25 ng	8	244.50 ± 35.95	10.96 ± 1.49	6.06 ± 1.39	2.79 ± 0.83
AP-5 25 ng	9	327.55 ± 62.53	12.75 ± 1.70	6.41 ± 0.87	4.37 ± 1.08
AP-5 50 ng	9	288.55 ± 37.86	14.97 ± 2.39	9.53 ± 1.87	3.06 ± 0.97
Experiment 2:					
Vehicle	8	254.75 ± 23.11	8.96 ± 1.02	4.83 ± 0.62	3.88 ± 0.95
DNQX 0.25 ng	9	338.55 ± 58.73	6.82 ± 1.30	5.16 ± 0.88	3.27 ± 0.99
DNQX 0.5 ng	8	241.25 ± 18.36	6.34 ± 0.81	2.69 ± 0.46	2.87 ± 1.03
DNQX 1 ng	8	249.87 ± 20.13	8.67 ± 1.71	4.56 ± 0.92	2.33 ± 0.58
Experiment 3: NMDA/DA					
Vehicle	9	206.01 ± 16.83	9.87 ± 1.29	4.38 ± 0.39	2.62 ± 0.90
SCH 6.25 ng	9	210.33 ± 18.86	9.28 ± 1.32	4.28 ± 0.66	2.40 ± 0.68
SULP 6.25 ng	8	177.37 ± 20.00	9.30 ± 1.40	4.01 ± 0.70	2.35 ± 0.72
AP-5 3.125 ng	9	196.77 ± 14.32	13.37 ± 2.04	4.64 ± 0.75	3.04 ± 0.78
AP-5+SCH	9	159.11 ± 22.32	7.46 ± 1.13	3.93 ± 0.63	2.29 ± 0.77
AP-5+SULP	8	195.87 ± 28.40	7.09 ± 0.98	5.29 ± 0.94	2.76 ± 0.68
Experiment 4: AMPA/DA					
Vehicle	9	210.88 ± 11.45	9.21 ± 0.87	4.65 ± 0.70	3.23 ± 0.47
SCH 6.25 ng	9	207.00 ± 14.97	8.54 ± 0.87	5.06 ± 0.70	1.99 ± 0.47
SULP 6.25 ng	9	160.11 ± 22.05	5.74 ± 0.87	3.83 ± 0.70	2.45 ± 0.47
DNQX 0.25 ng	9	235.66 ± 29.35	7.03 ± 0.87	5.27 ± 0.70	3.43 ± 0.47
DNQX+SCH	8	214.01 ± 17.77	7.86 ± 0.93	4.92 ± 0.75	2.50 ± 0.50
DNQX+SULP	9	198.44 ± 33.34	8.54 ± 0.87	2.79 ± 0.70	1.96 ± 0.47

Number of subjects (mice) per experimental group. Locomotor activity (mean number of sector crossed, ± SEM) after intra-VS drug infusion (S2–S6). Mean duration of contacts (in seconds, ± SEM) with the entire set of objects during habituation (S2–S4) sessions.

dose-dependent decrease of the exploration of DOs and did not affect the exploration of NDOs. The ANOVA showed a significant spatial novelty (DO/NDO) effect ($F_{1,38} = 56.99$, $P < 0.001$), a significant treatment effect ($F_{4,38} = 3.07$, $P = 0.0275$) and a significant treatment $\times$ spatial novelty effect ($F_{4,38} = 6.16$, $P < 0.001$). The *post hoc* comparison further revealed that, differently to vehicle- and lowest dose-infused mice (AP-5 3.125 ng/side), mice infused with the other AP-5 doses (6.25, 25 and 50 ng/side) did not spent a significantly higher amount of time re-exploring DO as compared with NDO.

Figure 3 also illustrates the effects of vehicle and AP-5 intra-VS infusion on reactivity to non-spatial novelty, expressed as the difference in time spent exploring the two different categories of objects (SO/NSO) in S6 and S5. The ANOVA revealed a non-significant treatment effect, a significant non-spatial novelty effect ($F_{1,38} = 141.690$, $P < 0.001$) and no effect of the interaction between the two factors. The *post hoc* comparison confirmed that, likewise control mice, all the animals infused with AP-5 significantly reacted to the novel object, whatever the dose of NMDA antagonist administered. These data demonstrate that VS NMDA receptor blockade reduces mice ability to react to a spatial change, without affecting other behavioral parameters.

Experiment 2: Intra-VS DNQX Infusions

Locomotor activity and habituation. Bilateral intra-VS infusion of the competitive AMPA antagonist did not alter the number of sectors crossed by mice (Table 1). This was confirmed by the ANOVA that did not reveal any significant treatment effect. DNQX infusion did not alter the exploratory activity during habituation. The ANOVA revealed a significant sessions effect ($F_{2,58} = 32.04$, $P < 0.001$) and no effect of treatment or a significant treatment $\times$ sessions interaction.

Reactivity to spatial and non-spatial novelty. Figure 4 shows the effect of bilateral intra-VS DNQX infusion on the re-exploration of spatial novelty (DO/NDO), expressed as the difference in time spent in contact with the two categories of objects in S5 minus S4. The ANOVA revealed a significant main spatial novelty effect (DO/NDO) ($F_{1,29} = 34.97$, $P < 0.001$), no significant treatment effect and a significant treatment $\times$ spatial novelty interaction ($F_{3,29} = 5.926$, $P = 0.002$). As shown by the *post hoc* comparison, both controls and mice infused with the lowest dose of DNQX (0.25 ng/side) renewed the exploration of DO category. In contrast, when infused at 0.5 and 1 ng/side, the DNQX treatment impaired the reactivity to spatial change (Figure 4).

The same figure depicts the effects of intra-VS DNQX infusion on reactivity to non-spatial novelty. The ANOVA revealed a significant treatment effect ($F_{3,29} = 5.58$, $P = 0.003$) as well as a significant object category (SO/NSO) effect ($F_{1,29} = 101.80$, $P < 0.001$) and a significant treatment $\times$ object category interaction ($F_{3,29} = 8.98$, $P = 0.002$). The *post hoc* comparison revealed that DNQX infusion dose-dependently reduced but not abolished the renewal of SO exploration. These findings demonstrate that VS blockade of AMPA receptors not only impairs detection

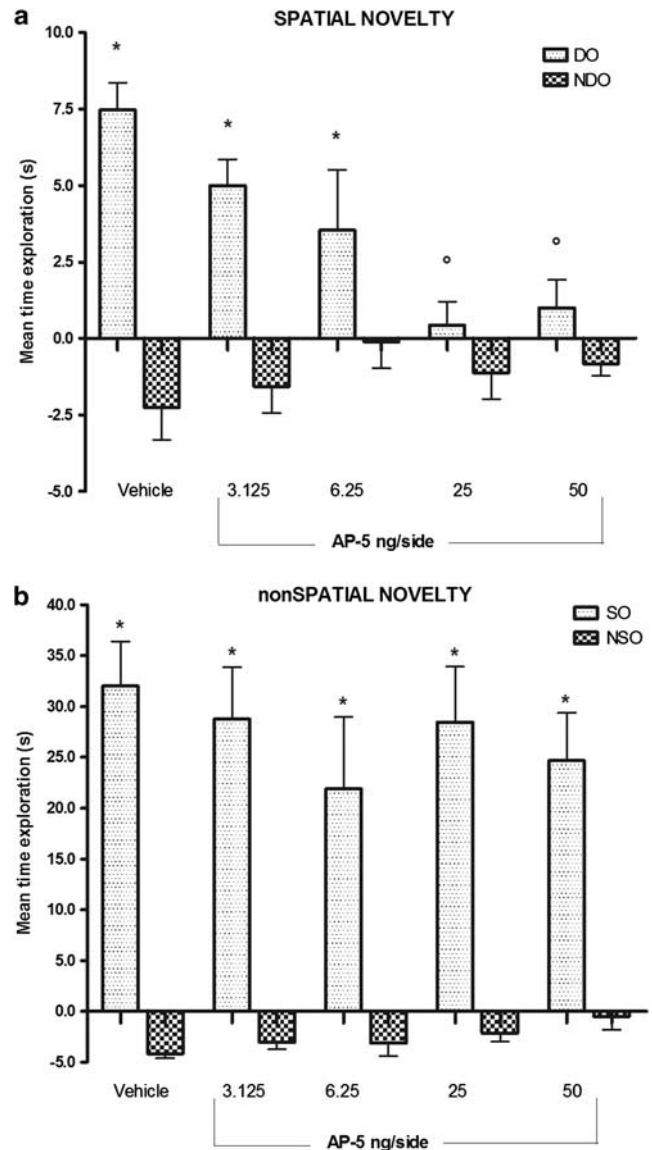


Figure 3 Experiment 1: ability to detect spatial (a) or non-spatial (b) novelty in ventral striatum (VS)-infused mice immediately before S2 with vehicle or AP-5 (3.125, 6.25, 25, 50 ng/side). (a) The time spent (mean $\pm$ SEM) in contact with displaced (DO) or non-displaced objects (NDO) categories in S5 minus the time spent exploring the same object category during the last habituation session (S4). (b) The time spent (mean $\pm$ SEM) in contact with substituted (SO) or non-substituted objects (NSO) in S6 minus the time spent exploring the same object category during the previous session (S5). * $P < 0.05$ DO/SO vs NDO/NSO; $^{\circ}P < 0.05$ DO vs vehicle DO.

of a spatial change, but also reduces the exploration of novel objects.

Experiment 3: NMDA/DA. Intra-VS Coinfusion of Ineffective Doses of AP-5 and SCH or SULP

Locomotor activity and habituation. Neither the separate infusion of AP-5, SCH or SULP, nor the combination between AP-5 and SCH or between AP-5 and SULP did alter the total number of sector crossed (S2–S6) (Table 1). The ANOVA did not revealed any significant effect. Moreover,

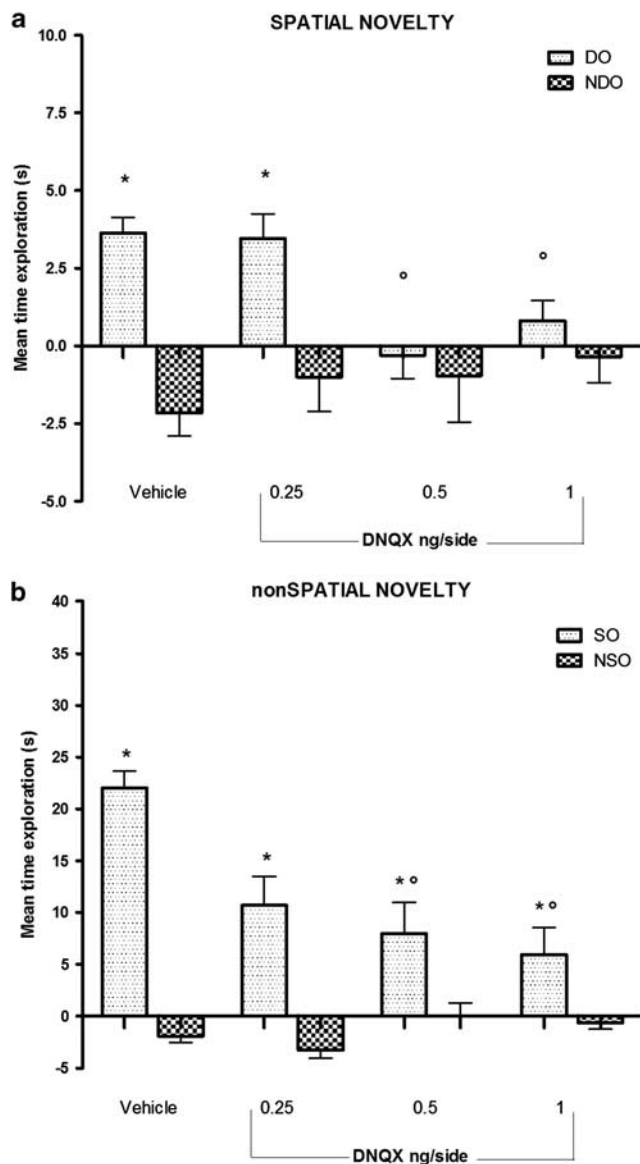


Figure 4 Experiment 2: ability to detect spatial (panel a) or non-spatial (panel b) novelty in ventral striatum (VS)-infused mice immediately before S2 with vehicle or DNQX (0.25, 0.5, 1 ng/side). Panel a depicts the time spent (mean $\pm$ SEM) in contact with displaced (DO) or non-displaced objects (NDO) categories in S5 minus the time spent exploring the same object category during the last habituation session (S4). Panel b depicts the time spent (mean $\pm$ SEM) in contact with substituted (SO) or non-substituted objects (NSO) in S6 minus the time spent exploring the same object category during the previous session (S5). * $P < 0.05$ DO/SO vs NDO/NSO; ° $P < 0.05$ DO vs vehicle DO (displaced objects). NDO, non-displaced object.

the intra-VS infusion of single doses of AP-5, SCH or SULP confirmed their inefficacy in altering the exploratory activity exhibited by the animals on the whole set of the objects during habituation sessions (Table 1). The ANOVA revealed only a significant effect of sessions ($F_{2,92} = 95.90$, $P < 0.0001$).

Reactivity to spatial and non-spatial novelty. In the Figure 5 are illustrated the effects of NMDA/DA receptor

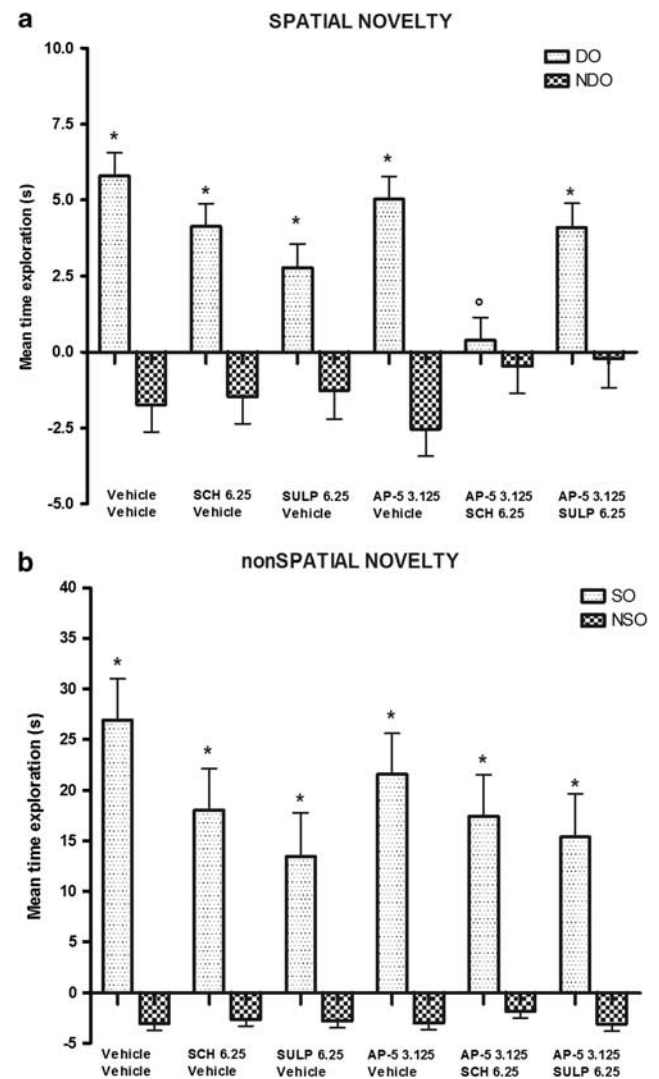


Figure 5 Experiment 3: ability to detect spatial (a) or non-spatial (b) novelty in ventral striatum (VS)-infused mice immediately before S2 with the following drugs: vehicle, SCH 23390 (6.25), SULP (6.25), AP-5 (3.125), AP-5 (3.125) + SCH (6.25), AP-5 (3.125) + SULP (6.25) (ng/side). (a) The time spent (mean $\pm$ SEM) in contact with displaced (DO) or non-displaced objects (NDO) categories in S5 minus the time spent exploring the same object category during the last habituation session (S4). (b) The time spent (mean $\pm$ SEM) in contact with substituted (SO) or non-substituted objects (NSO) in S6 minus the time spent exploring the same object category during the previous session (S5). * $P < 0.05$ DO/SO vs NDO/NSO; ° $P < 0.05$ DO vs vehicle DO.

interaction following bilateral intra-VS coinfusion of AP-5 plus SCH and AP-5 plus SULP, expressed as difference in the time spent in the exploration of the two categories of objects in S5 and S4. As shown, control animals reacted to spatial novelty by re-exploring DO in S5, and decreasing the exploration of NDO. Intra-VS infusions of ineffective doses of AP-5, SCH and SULP (3.125, 6.25 and 6.25 ng/side, respectively) as well as coinfusions of AP-5 and SULP did not impair the ability of mice to detect spatial novelty (Figure 5). Conversely, the intra-VS coinfusion of ineffective doses of AP-5 + SCH suppressed the re-exploration of DO. The ANOVA showed a significant spatial novelty (DO/NDO) effect ($F_{1,46} = 141.67$, $P < 0.0001$) and a significant DA

treatment $\times$ spatial novelty interaction ($F_{2,46}=10.12$, $P<0.001$), and a significant DA $\times$ NMDA $\times$ spatial novelty interaction ($F_{2,46}=3.86$, $P<0.02$). As showed in Figure 5, the *post hoc* comparison revealed the significant difference between object category for vehicle controls and mice infused with ineffective doses of AP-5, SCH, Sulp as well as for animals receiving coinfusion of AP-5 and Sulp (NMDA/D2 receptor interaction), while no significant difference in the exploration of the two object categories was detected in animals coinfused with ineffective doses of AP-5 and SCH (NMDA/D1 receptor interaction).

The Figure 5 also shows the effects of the separate infusion of NMDA, D1 and D2 antagonist on the reactivity to non-spatial novelty (SO/NSO), expressed as the difference in time spent exploring the two categories of objects in S6 and S5. The ANOVA revealed a significant effect of non-spatial novelty ($F_{1,46}=137.20$, $P<0.0001$) but not other significant effect. The *post hoc* comparison revealed a significant difference in the re-exploration values for the two object categories (SO/NSO) for all the experimental groups. Thus, demonstrating that the specific impairment observed with coinfusions of NMDA/D1 receptors antagonists in the VS is selective to spatial information.

Experiment 4: AMPA/DA Intra-VS Coinfusion of Ineffective Doses of DNQX and SCH or Sulp

Locomotor activity and habituation. Also in this case neither of the treatments did alter the total number of sector crossed (S2–S6) (Table 1). The two-way ANOVA did not show a significant effect. The intra-VS infusion of the ineffective doses of the AMPA antagonists as well as the D1 or D2 antagonists confirmed their inefficacy in altering the exploratory activity displayed by the animals on the whole set of the objects during habituation sessions (Table 1). Table 1 shows that the coinfusion of DNQX and SCH or Sulp did not alter the exploratory activity during habituation sessions (S2–S4). The ANOVA revealed only a significant sessions ($F_{2,94}=26.40$, $P<0.001$) and AMPA treatment effect ($F_{1,47}=4.19$, $P=0.04$).

Reactivity to spatial and non-spatial novelty. In the Figure 6 are illustrated the effects of AMPA/DA receptor interaction following bilateral intra-VS coinfusion of ineffective doses of DNQX plus SCH or Sulp, expressed as difference in the time spent in the exploration of the two categories of objects in S5 and S4. Vehicle control animals reacted to spatial novelty by re-exploring DO in S5, while decreasing the exploration of NDO in S5. Intra-VS separate blockade of AMPA, D1 and D2 receptors (by the infusion of DNQX, SCH and Sulp at 0.25, 6.25 and 6.25 ng/side, respectively) did not impair the ability of the animals to detect the spatial change, as occurred in S5. Interestingly, while the coinfusion of DNQX and SCH did not alter the exploration of spatial novelty, intra-VS coinfusion of DNQX and Sulp suppressed the exploration of DOs and slightly increased the exploration of NDOs. The two-way ANOVA revealed a significant spatial novelty effect ($F_{1,47}=118.86$, $P<0.0001$), a significant interaction between DA treatment and spatial novelty ($F_{2,47}=7.28$, $P=0.001$) as well a significant AMPA $\times$ DA $\times$ spatial novelty interaction ($F_{2,47}=6.75$, $P=0.002$). The *post hoc* comparison further

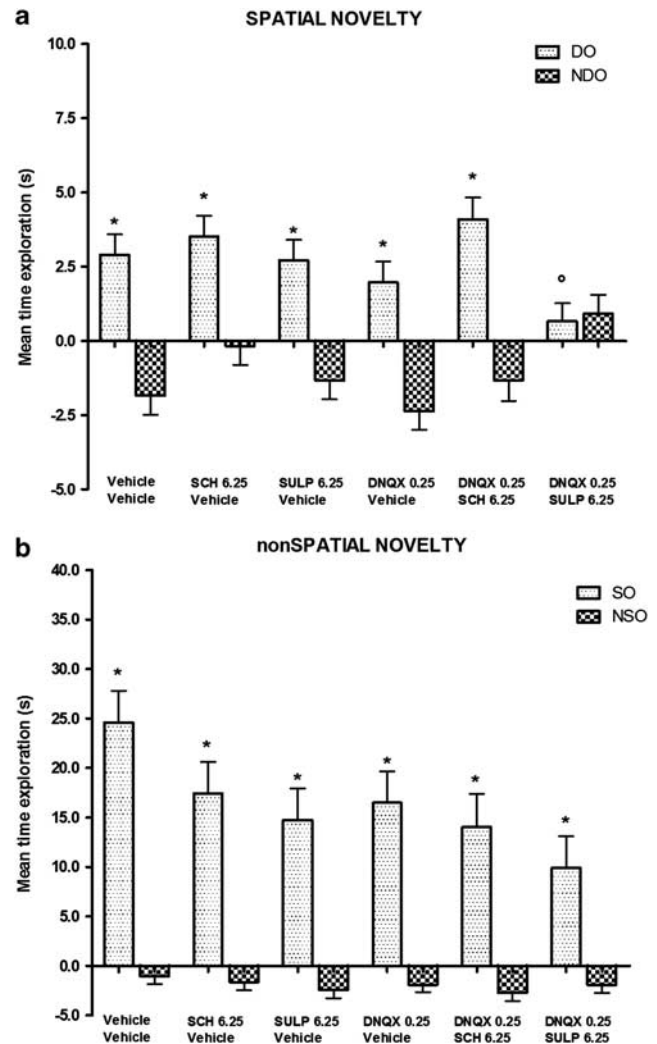


Figure 6 Experiment 4: ability to detect spatial (a) or non-spatial (b) novelty in ventral striatum (VS)-infused mice immediately before S2 with the following drugs: vehicle, SCH (6.25), Sulp (6.25), DNQX (0.25), DNQX (0.25) + SCH (6.25), DNQX (0.25) + Sulp (6.25) (ng/side). (a) The time spent (mean $\pm$ SEM) in contact with displaced (DO) or non-displaced objects (NDO) categories in S5 minus the time spent exploring the same object category during the last habituation session (S4). (b) The time spent (mean $\pm$ SEM) in contact with substituted (SO) or non-substituted objects (NSO) in S6 minus the time spent exploring the same object category during the previous session (S5). * $P<0.05$ DO/SO vs NDO/NSO; ° $P<0.05$ DO vs vehicle DO.

revealed (Figure 6) a significant difference between the two object categories (DO and NDO) in all the experimental groups except for mice coinfused with ineffective doses of DNQX and Sulp (AMPA/D2 receptor interaction).

In Figure 6 are also shown the effects of the separate infusion of AMPA, D1, and D2 antagonist as well as of the AMPA/D1 (DNQX and SCH combination) and AMPA/D2 (DNQX and Sulp combination) receptor interaction on the reactivity to non-spatial novelty (SO/NSO), expressed as the difference in time spent exploring the two categories of objects in S6 and S5. All groups selectively re-explored the SO category. The two-way ANOVA revealed only a significant object category ($F_{1,47}=223.53$, $P<0.001$) and DA treatment effect ($F_{2,47}=3.25$, $P=0.04$), but not

significant AMPA treatment ($F_{1,47} = 3.32$, $P = \text{ns}$) and AMPA/DA $\times$ object category effect ($F_{2,47} = 0.42$, $P = \text{ns}$). The *post hoc* comparison revealed a significant difference between the re-exploration times for the two object categories (SO/NSO) in all groups. These findings demonstrate a specific interaction between AMPA and D2 receptors in the VS in the modulation of spatial information processing.

DISCUSSION

The results described here demonstrate that colocalization of ionotropic GLUergic and DAergic receptors on the same MSN in the VS entails a functional significance for the encoding of spatial information. First we report that independent and selective intra-VS NMDA and AMPA receptors blockade impairs, in a dose-related manner, the ability to detect a spatial change. Then, we provide evidence of a receptor-specific functional coupling between different DA and GLU receptor subtypes in the modulation of VS behavioral output. We found that concomitant infusion of ineffective doses of D1/NMDA but not D2/NMDA (Figure 5) and D2/AMPA but not D1/AMPA (Figure 6) receptor antagonists specifically impairs the acquisition of spatial information, thus supporting the view of a synergistic interaction between heterogeneous receptor subtypes.

Ionotropic Glutamate Receptor are Involved in Different Degree in Spatial and Non-spatial Information Processing

Several pieces of evidence obtained by receptors blocking studies corroborate the idea that VS NMDA-Rs are required to build up representations of the spatial environment (Maldonado-Irizarry and Kelley, 1995; Adriani *et al.*, 1998; Usiello *et al.*, 1998; Smith-Roe *et al.*, 1999). By the use of AP-5 infusions over a wide range of doses, the present study provides evidence that VS NMDA-Rs are necessary to acquire and update the spatial information. The escalating dose regimen of AP-5 infusions disclosed a linear dose-dependent functional involvement of NMDA-Rs in the detection of a spatial change that did not involve habituation development or reactivity to non-spatial novelty (Figure 3). This observation confirms previous findings demonstrating that AP-5-induced impairment in object recognition occurs only at higher doses (Sargolini *et al.*, 2003b), suggesting that NMDA-Rs, at these dose range, might have a selective role in the processing of spatial information.

In parallel, this study also provides evidence of the involvement of AMPA-Rs in the detection of a novel spatial configuration (Figure 4). Similar to NMDA-Rs, the blockade of AMPA-Rs before training (before S2) dramatically disrupts the ability to react to the spatial change (S5) (Figure 4). At difference with NMDA-R blockade, however, the infusion of the AMPA-R antagonist also reduced the exploration of non-spatial novelty. It should be mentioned that the drug did not affect novel object exploration in S2, habituation to the overall set of objects or the pattern of locomotor activity, making it difficult to attribute the effects

observed to a generalized impairment in exploratory activity.

Overall, the present findings confirm previous evidence that glutamate receptors located in the VS have a role in the short-term processing of spatial information (Ferretti *et al.*, 2007). The VS receives dense GLUergic excitatory projections from the hippocampus, amygdala, thalamus, and entorhinal, perirhinal and prefrontal cortices (Groenewegen *et al.*, 1987; Groenewegen *et al.*, 1991; Berendse *et al.*, 1992). In light of the role hypothesized for hippocampus and perirhinal cortex in spatial learning and object recognition, respectively (Murray and Bussey, 1999; Buckley, 2005), it seems conceivable that the effects of intra-VS GLU-Rs antagonists on spatial and non-spatial novelty detection might be due to impaired transmission of information from these structure (Sargolini *et al.*, 1999). Nevertheless, in our study, the independent blockade of NMDA- and AMPA-Rs exerted a dissimilar impact on processing of spatial and non-spatial information. In fact, while in line with previous reports (Roulet *et al.*, 2001; Sargolini *et al.*, 2003a; Sargolini *et al.*, 2003b; Ferretti *et al.*, 2007) intra-VS AP-5 infusion selectively impaired the detection of a spatial change, AMPA-Rs blockade resulted in a more extended impairment affecting also the exploration of non-spatial novelty. In light of the suggested role of the VS as an interface between the limbic and the motor system (Mogenson *et al.*, 1980) that has a role in the action selection (Nicola, 2007), it could be suggested that the difference observed after manipulation of the two receptor subtypes might depend on the degree of further integration needed for information processed upstream to this region in order to exert control over the behavioral response. In this framework, it could be speculated that AMPA-Rs activation followed by the intracellular signaling mediated by NMDA-Rs activation is a better suitable process for reaction to a spatial change.

Single Subtypes of Ionotropic Glutamate and Dopamine Receptors Functionally Interact to Encode Spatial Information

We have previously provided evidence that VS D1- and D2-Rs are both involved, in different degree, in the acquisition of spatial information (Coccurello *et al.*, 2000). In light of the close apposition between DA- and GLU-Rs reported on VS MSN and the functional interaction between the two classes of receptors in the modulation of MSN activity (Cepeda *et al.*, 1993; Cepeda and Levine, 1998; Flores-Hernandez *et al.*, 2002), we investigated possible functional interactions between these two receptor system in spatial information processing. To do so, we used *per se* ineffective dose of either D1- or D2-Rs antagonists (Coccurello *et al.*, 2000) in association with sub-threshold doses of AMPA- or NMDA-Rs antagonists as assessed in the previous two experiment.

By intra-VS coinfusion of ineffective doses of NMDA/D1 receptor antagonists, this study provides the first evidence of the importance of NMDA/D1-Rs functional interaction for the detection of a spatial novelty. Indeed, our findings demonstrate that the combination of ineffective doses of AP-5 and SCH 23390 selectively impaired the detection of spatial change. Interestingly, no change was observed on the habituation pattern of the animals or on the detection of the

novel object in S6, thus, demonstrating that the effect at these doses was specific to spatial information processing.

In the next series of experiments, we found that coinjection of ineffective doses of AMPA- and D2-Rs antagonists produced a marked impairment of spatial learning. Also in this case the increased exploration of the DOs observed in S5 was not paralleled by changes on the other behavioral parameters recorded (habituation, novel object detection). Remarkably, the impairment observed after coadministration of AP-5 and SCH 23390 or DNQX and sulpiride was absent, when AP-5 was administered in combination with sulpiride or DNQX in combination with SCH 23390. Therefore, this set of experiments supports the susceptibility of VS spatial information processing to concurrent blockade of DA and GLUergic receptors. Moreover, these experiments provide evidence for the existence of preferential NMDA/D1 and AMPA/D2, but not NMDA/D2 or AMPA/D1, functional interaction in the modulation of short-term spatial information processing in the VS.

VS Gating and Glutamate–Dopamine Receptors Interplay in Spatial Information Processing: Some Final Functional Remarks

The interplay between DA- and Glu-Rs in the striatal complex is supported by several structural, physiological, and biochemical evidence (for review see Cepeda and Levine, 1998). From a behavioral point of view most studies focused on locomotor activity, pointing to an interaction between the two receptor systems in regulating VS functional output. Nevertheless, still elusive remains the exact relationship among the different receptors subtypes. For example, both D1 and D2 antagonist impair systemic MK-801-induced locomotor activity (Ouagazzal *et al.*, 1993). Similarly, rotational behavior induced by AMPA injections in the VS is blocked by a mixed D1/D2-R antagonist, thus independently of the receptor subtype (Ikeda *et al.*, 2003). A further issue is linked to the outcome of the concomitant stimulation of ionotropic GLUergic and DAergic receptor subtypes on locomotor activity. Intriguing in this regard is the observation that VS coinjections of different Glu- and DA-Rs agonists and antagonists can induce both positive and negative modulations of locomotor activity (David *et al.*, 2004; David *et al.*, 2005), thus, indicating that the effects of DA and Glu interactions on VS output depend on the level of interaction (ie, pre- or post-synaptic level) (David *et al.*, 2004; David *et al.*, 2005). Finally, Burns *et al.* (1994) comparing the effects induced by different GLU-Rs agonists and antagonists, alone or in combination with amphetamine, on locomotor activity and conditioned responding suggested that the interplay between the two systems might critically depends upon the behavioral paradigm tested.

Short-term spatial information processing would be expected to depend upon sustained electrical activity or changes in synaptic efficacy. In this perspective the effects evident after concurrent NMDA/D1-Rs blockade are coherent with the electrophysiological findings demonstrating that NMDA-mediated currents are enhanced by activation of D1 receptors on MSNs (Nicola *et al.*, 2000). Moreover, as not only the NMDA subtype, but also group I metabotropic GLU (mGLU) receptors are positively coupled with D1-mediated locomotor activity (Rouillon *et al.*, 2008), it is

possible to hypothesize that concomitant blockade or activation of group I mGLU/D1-Rs might impair spatial information processing within the VS in a similar manner. As regards to the effects of AMPA/D2 receptors blockade, data available in the literature are more contradictory. The stimulation of AMPA-Rs has been shown to potentiate the motor activity elicited by the activation of post-synaptic D2-Rs (David *et al.*, 2005), thus mirroring the effects observed in the present study on the reactivity to spatial change. On the contrary, cellular and electrophysiological studies suggest that AMPA and D2 receptors might be functionally coupled in nonreciprocal manner, so that D2 stimulation depresses AMPA-mediated responses (Cepeda *et al.*, 1993; Levine *et al.*, 1996; Flores-Hernandez *et al.*, 2002). Although supporting the view of a preferential AMPA/D2-Rs interplay, the direction of this interaction appears opposite to the one expected on the basis of the results presented in this study or those observed on locomotor activity (David *et al.*, 2005). Nevertheless, these observations might be reconciled in light of the particular role that, in this context, D2 receptors can have in the optimization of signal-to-noise ratio. In this framework, it seems conceivable that either concomitant stimulation or blockade of these receptor subtype may produce similar information processing deficit.

Different models have been suggested to describe DA-Glu interplay in the striatal complex. Some observations point to reciprocal pre-synaptic interaction (Wan *et al.*, 1995), other findings suggest that the interactions might depend on the effects at a post-synaptic level and on the activity of the MSNs (Ouagazzal *et al.*, 1994; Mele *et al.*, 1998). These models are not necessarily mutually exclusive, and it seems conceivable that both the levels of interactions might occur (David *et al.*, 2005). It should also be considered that the VS is characterized by a profound biochemical and neuroanatomical diversity that reflects on the functional output of the structure. This is demonstrated by the different effects induced by the manipulation of the two major components of the VS, the core and the shell, in different behavioral paradigms: locomotor activity (Pulvirenti *et al.*, 1994), prepulse-inhibition (Wan and Swerdlow, 1996), novelty exploration (Maldonado-Irizarry and Kelley, 1994), as well as in learning and memory (Maldonado-Irizarry and Kelley, 1995; Smith-Roe *et al.*, 1999; Klein *et al.*, 2004; Ito *et al.*, 2008; Managò *et al.*, 2009). This regional heterogeneity reaches a further level of complexity when considering DA-Glu interactions that show regional specificity across the VS subregions (Pulvirenti *et al.*, 1994; Wan and Swerdlow, 1996). Although the investigation of regional differences in DA- and Glu-Rs interactions was not the purpose of this study and other experiments would be needed to properly address this issue, it seems conceivable that response to spatial and object novelty in the VS is modulated by DA-Glu interplay in a receptor subtype- and region-dependent manner.

In conclusion, the present data support the view that besides hippocampus (Kumaran and Maguire, 2007) and prefrontal cortex (Matsumoto *et al.*, 2007; Rinaldi *et al.*, 2007) the VS is also involved in spatial information processing. Indeed, VS NMDA and AMPA-Rs blockade impaired the ability to react to a spatial novelty. Furthermore, our data demonstrate for the first time that preferential NMDA/D1 or AMPA/D2 receptor–receptor

functional interaction is required in the VS for shaping the reactivity to spatial novelty. In this view, the functional interaction between specific subtypes of ionotropic glutamate and D1/D2 receptors in VS may dictate the outcome of salient information processing.

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DISCLOSURE

The authors declare no conflict of interest.

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