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Role of dopamine and glutamate receptors in cocaine-induced social effects in isolated and grouped male OF1 mice

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Abstract

Cocaine administration in paired male mice decreases social contacts as well as increases avoidance and flee elements. As dopamine (DA) and glutamate seem to be involved in some of cocaine's effects, an attempt was made to assess whether a range of associated receptors influenced the social impacts of this drug of abuse. The NMDA antagonist memantine (10 and 40 mg/kg); the AMPA antagonist CNQX (1 and 20 mg/kg); the DA release inhibitor CGS 10746b (2 and 8 mg/kg): the DA D1 antagonist SCH 23390 (0.05 and 0.5 mg/kg); and the DA D2/D3 antagonist raclopride (0.03 and 0.3 mg/kg) were administered prior to 25 mg/kg of cocaine and behaviour was evaluated during an encounter between an experimental and a standard opponent in a neutral cage for 10 min. Memantine reverts cocaine-induced social withdrawal and the increase in avoidance and flee, CNQX being effective only in these latter actions. On the other hand, SCH 23390 counteracts the social as well as the defensive action of cocaine, raclopride being effective only in blocking the cocaine-induced increase in avoidance and flee behaviours. In conclusion, although both neurotransmitter systems are involved in the effects of cocaine on social behaviour, NMDA and D1DA receptors seem to have an important role.

Keywords: Memantine; CNQX; CGS 10746B; SCH 23390; Raclopride; Cocaine; Mice; Social interaction

1. Introduction

Cocaine administration induces a complex series of changes in social behaviour, however, there is no agreement regarding its specific actions on aggression. Several studies report increased irritability and aggression in humans after a prolonged use of high doses of cocaine (Bukstein, 1996; Denison et al., 1997; Gordon et al., 1996; LeSage et al., 1999; McCormick and Smith, 1995; Miller et al., 1991). On the contrary, a number of authors have not found any correlation between cocaine intake or its withdrawal and an increase in aggressive behaviours (Dhossche, 1999; Moeller et al., 1997). These discrepancies are equally observed when studying the link between cocaine and aggression with animal models. In mice and rats, no changes or a reduction in threat and attack has been found after different cocaine doses (Miczek and O'Donnell, 1978; Miczek, 1979). Conversely, increases in attack have been found in other studies using mice, rats or primates (Emley and Hutchinson, 1983; Hadfield et al., 1982; Filibeck et al., 1988; Long et al., 1996). In addition to altering aggressiveness, cocaine induces other changes in interactive social behaviours. An increase in avoidance and flee has been generally reported (Blanchard and Blanchard, 1999). On the other hand, there is a general consensus regarding the reduction in social contacts with conspecifics observed after cocaine administration (Darmani et al., 1990; Rademacher et al., 2002).

In a recent work performed in our laboratory (Estelles et al., 2004), we evaluated the complex actions of cocaine on social behaviour of mice exposed to different housing situations (isolated- or group-housed) using confrontations between two male mice in a neutral area, and administering different doses of cocaine (6, 25 and 50 mg/kg) given in a single or binge pattern (three doses in 24 h). No increases in aggression were observed in any situation tested. Instead, the two highest doses of cocaine, both in a single and binge administration, decreased aggressive behaviours in isolated mice, no changes being found in grouped animals. In both types of animals, cocaine increased defensive elements (avoidance/flee) and abolished social

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contacts. These results suggest that cocaine, besides presenting an antiaggressive action, also exerts an anxiogenic-like effect.

Among brain neurotransmitters, dopamine (DA) is the most extensively implicated in the mechanism of drug addiction, not only as the substrate of psychostimulant reward, but more generally as a substrate of drug-related learning and neuroadaptation (Di Chiara et al., 2004). Acute or binge administration of cocaine induces a release of DA in the Nucleus Accumbens (N Acc) (Broderick et al., 1993; Zhang et al., 2001), and an increase of its turnover during cocaine self-administration in this and other brain structures, such as the ventral pallidum or lateral hypothalamus (Smith et al., 2003). Both the D1 and D2 DA family receptors seem to be involved in cocaine effects. Administration of the D1 DA receptor antagonist SCH 23390 alone or in combination with a D2 antagonist prevents cocaine-induced conditioned place preference (CPP) (Baker et al., 1998; Liao et al., 1998). Reinstatement of cocaine-induced CPP after extinction either after a priming injection of cocaine or conditioned cues is also blocked with D1 and D2 DA antagonists (Sanchez et al., 2003; Sun and Rebec, 2005). The blockade of D1 receptors also prevents the sensitised response to cocaine-conditioned rewarding effects (Shippenberg and Heidbreder, 1995). Hyperdefensiveness observed in animals chronically treated with cocaine is completely blocked after administration of the D2 DA antagonist sulpiride (Filibeck et al., 1988).

Another brain neurotransmitter, glutamate, is also implicated in psychostimulant actions. Acute and chronic administration of cocaine in rats significantly elevates glutamate levels in various limbic brain areas (Bell et al., 2000). Recent data indicate that activation of glutamatergic afferents from the amygdala and prefrontal cortex is critical in the expression of addictive behaviours. Using the reinstatement model of relapse, regardless of the stimulus modality, there is a dependence on glutamate neurotransmission in the prefrontal cortex (Capriles et al., 2003; McFarland et al., 2003; Shalev et al., 2002). Ionotropic glutamate receptors in the N Acc influence self-administration of cocaine and relapse (Cornish and Kalivas, 2000), cocaine-induced hyperlocomotion (Witkin, 1993) and behavioural sensitisation (Wolf, 1998). Cocaine-induced CPP is also abolished after the blockade of glutamatergic neurotransmission (Cervo and Samanin, 1995; Kim et al., 1996; McGeehan and Olive, 2003; Slusher et al., 2001).

The aim of the present work is to assess the role played by dopamine and glutamate neurotransmission in the social behavioural effects induced by cocaine administration in male mice. To study DA transmission, drugs acting on different DA receptors or those inhibiting its release have been used. The D1 DA receptor antagonist SCH 23390, although exerting a slight effect on the D2 receptor, is considered to be a specific D1 receptor antagonist (Hyttel, 1983). To evaluate the role of D2 DA receptors, a highly selective D2 receptor antagonist, raclopride, has been used (de Paulis et al., 1986), although it also acts as a D3 receptor antagonist. Finally, a DA release inhibitor CGS 10746B, which does not alter DA metabolism or occupy DA receptors, was additionally employed (Altar et al., 1986). Glutamate neurotransmission was studied through two of its receptors, the NMDA and the AMPA. To this purpose, memantine, a low affinity noncompetitive NMDA receptor antagonist was used and also CNQX (6-cyano-7-nitroquinoxaline-2,3-dione), the competitive antagonist which acts on the glutamate recognition site of AMPA receptors (Witkin et al., 2003). The doses have been chosen on the basis of previous studies performed in our and other laboratories (Blanchard and Blanchard, 1999; Estelles et al., 2004; Felip et al., 2001; Itzhak and Martin, 2002; Miczek et al., 1999; Rodriguez-Arias et al., 1998, 1999, 2002; Sukhotina and Bespalov, 2000) except for CNQX, which were tested prior to this study. The low dose was behaviourally inactive for all the compounds and the highest presented a potent antiaggressive effect, except for CNQX, which did not have any antiaggressive action. Previous reports have shown that certain environmental conditions can alter the reinforcing effects of drugs like cocaine (LeSage et al., 1999). Many of these stimuli, such as the housing conditions of the animals, also produce changes in DA function (Bowling et al., 1983; Lapiz et al., 2001). To assess how housing conditions could affect the action of the drugs studied, animals were housed in groups or in isolation prior to the behavioural test.

2. Materials and methods

2.1. Subjects

Four hundred and eighty male mice of the OF1 strain (CHARLES RIVER, Barcelona, Spain) were used in this study. The animals were aged 42 days on arrival at the laboratory, and were housed under standard conditions with constant temperature (21±2 °C), a reversed light schedule (white lights on 19:30–07:30 h), and food and water available ad libitum (except during the behavioural test). Tests took place when animals were aged between 63 and 70 days. Half of the mice were used as experimental animals, and the remainder used as standard opponents. Experimental animals ($n=240$) were either housed in groups of four (cage size $28\times28\times14.5$ cm) or individually (cage size $23\times13.5\times13$ cm). Procedures involving mice and their care conformed to national, regional and local laws and regulations, and are in accord with the European Communities Council Directives (86/609/EEC, 24 November 1986).

2.2. Drugs

Cocaine hydrochloride (Laboratorios Alcaliber S.A., Madrid, Spain), memantine (Laboratorios Sigma-Aldrich Química, Madrid, Spain), CNQX (Laboratorios Sigma-Aldrich Química, Madrid, Spain), CGS 10746B (Novartis Pharmaceuticals Corporation, Summit, NJ, USA), SCH 23390 (Schering Plough, Madrid, Spain), and raclopride (Astra Laboratories, Södertälje, Sweden) have been used in these studies. All the compounds were diluted in physiological saline (0.9% NaCl), which was also used as vehicle (0.1 mg/ml), at a constant volume (10 ml/kg) and administered i.p. The DA and glutamate antagonists were administered 10 min before cocaine, which was injected 20 min before the

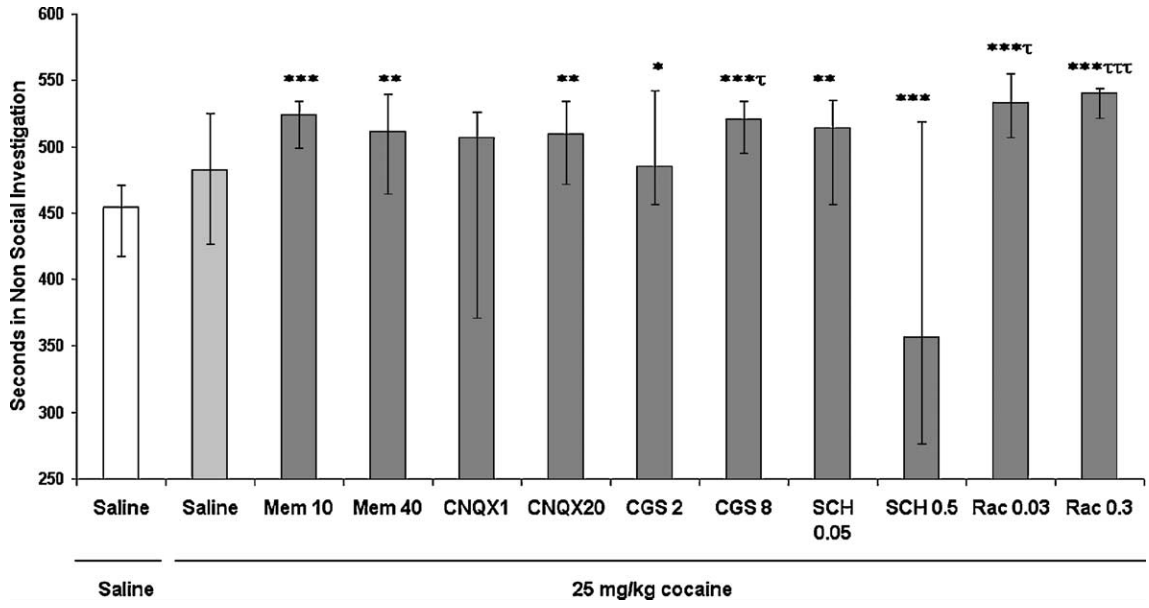

Fig. 1. Medians of accumulated times (in seconds) with percentile ranges of time spent in *Non Social Investigation* in *grouped* animals treated with 25 mg/kg of cocaine plus glutamate and dopamine antagonists. Differs on two-tailed Mann–Whitney *U*-test from Saline+Saline group * $p<0.05$; ** $p<0.02$; *** $p<0.001$; and from Saline+Cocaine group τ $p<0.02$; τττ $p<0.001$.

behavioural test. There were 10 animals in each treatment group.

2.3. Procedure and apparatus

The standard opponents were made temporarily anosmic by intranasal lavage with 4% zinc sulphate solution 1 day before testing (Smoothy et al., 1986). This kind of opponent is able to face attack but never initiates such behaviour, since it cannot perceive a pheromone present in the urine of the experimental animals which is considered to be a cue for eliciting aggressive behaviour in mice with a normal sense of smell (Brain et al., 1981; Mugford and Nowell, 1970). Tests consisted of an experimental animal and a standard opponent confronting each other in a neutral cage ($61 \times 30.5 \times 36$ cm) for 10 min, with 1 min of adaptation before the encounter. This confrontation was carried out 30 min after drug administration (20 min after cocaine injection) and videotaped under white illumination. The videotapes were analysed using a PC computer and a custom-developed programme (Brain et al., 1989) that facilitated estimation of times allocated to different broad functional categories of behaviour: non-social exploration; social inves-

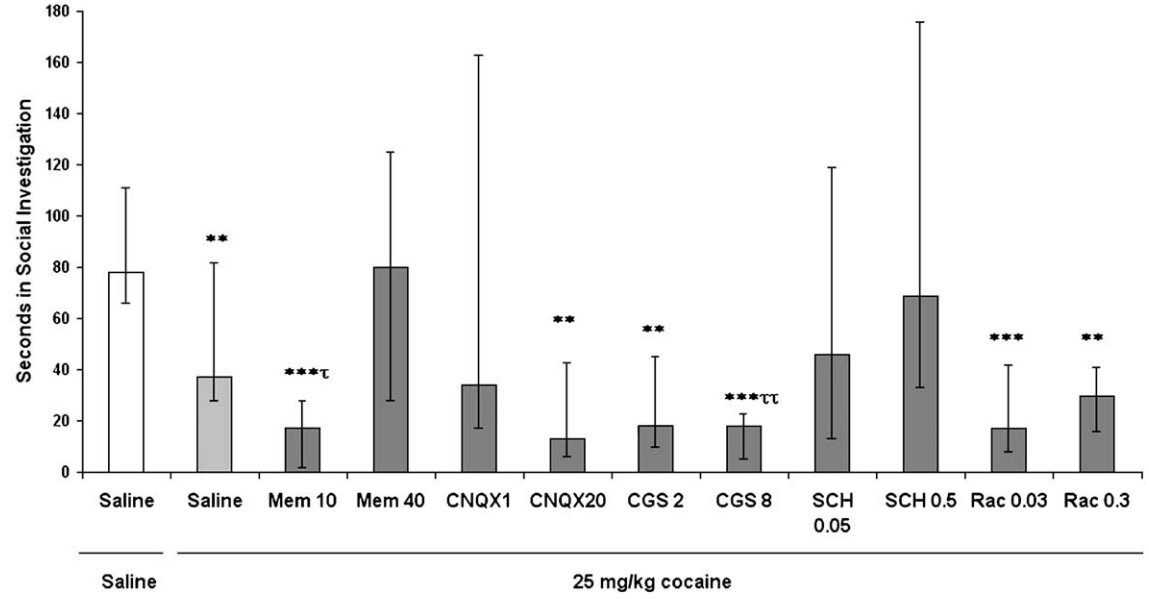

Fig. 2. Medians of accumulated times (in seconds) with percentile ranges of time spent in *Social Investigation* in *grouped* animals treated with 25 mg/kg of cocaine plus glutamate and dopamine antagonists. Differs on two-tailed Mann–Whitney *U*-test from Saline+Saline group ** $p<0.02$; *** $p<0.001$; and from Saline+Cocaine group τ $p<0.02$; ττ $p<0.01$.

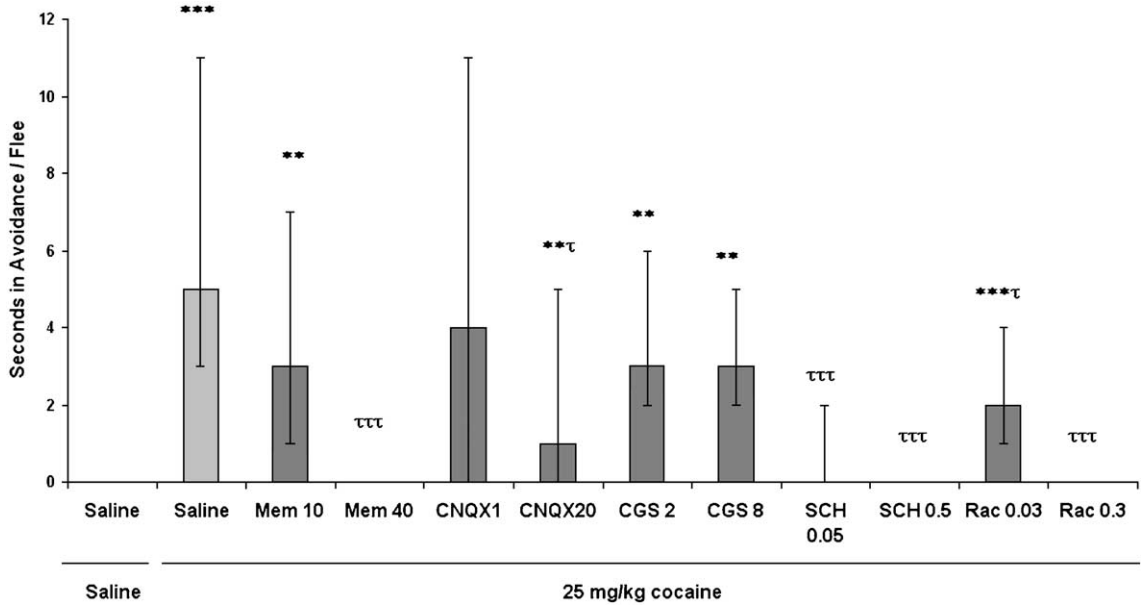

Fig. 3. Medians of accumulated times (in seconds) with percentile ranges of time spent in *Avoidance and Flee* in *grouped* animals treated with 25 mg/kg of cocaine plus glutamate and dopamine antagonists. Differs on two-tailed Mann–Whitney *U*-test from Saline+Saline group ** $p<0.02$; *** $p<0.001$; and from Saline+Cocaine group τ $p<0.02$; τττ $p<0.001$.

tigation; threat; attack; and avoidance/flee; each of which included a series of different postures and elements. A more detailed description can be found in Rodriguez-Arias et al. (1998).

2.4. Statistical analyses

Data of the social encounters were initially analysed using the Kruskal–Wallis test. For the behavioural categories in which this test was significant, differences between groups were examined using the two-tailed Mann–Whitney *U*-test.

3. Results

3.1. Effect of dopamine and glutamate antagonists on cocaine-induced behavioural changes in group-housed animals

Time spent by mice in *Non Social Exploration* (Kruskal–Wallis $p<0.001$) was increased in groups treated with cocaine plus memantine ($p<0.001$ for Coc-Mem10 and $p<0.02$ for Coc-Mem40), the high dose of CNQX ($p<0.02$), CGS ($p<0.05$ for Coc-CGS2 and $p<0.001$ for Coc-CGS8), the low dose of SCH ($p<0.02$), and raclopride ($p<0.001$) in

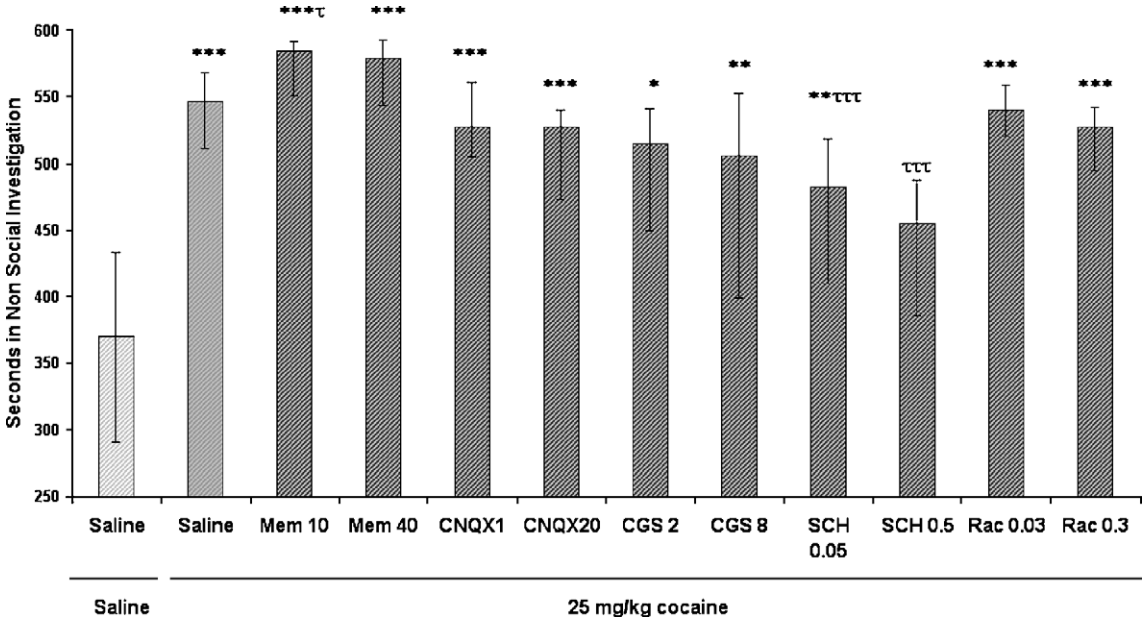

Fig. 4. Medians of accumulated times (in seconds) with percentile ranges of time spent in *Non Social Investigation* in *isolated* animals treated with 25 mg/kg of cocaine plus glutamate and dopamine antagonists. Differs on two-tailed Mann–Whitney *U*-test from Saline+Saline group * $p<0.05$; ** $p<0.02$; *** $p<0.001$; and from Saline+Cocaine group τ $p<0.02$; τττ $p<0.001$.

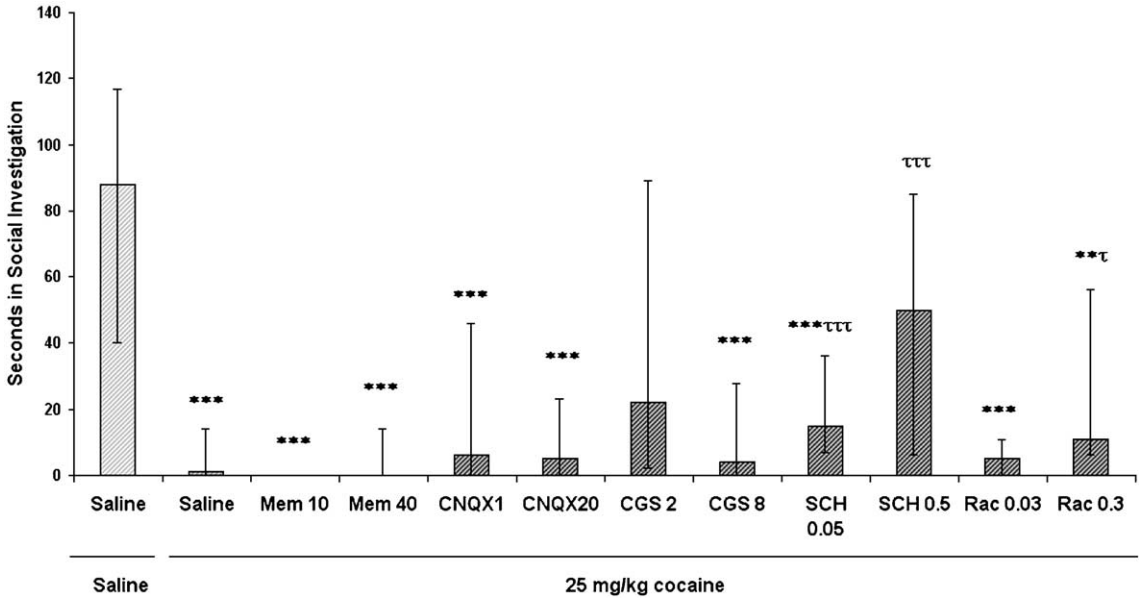

Fig. 5. Medians of accumulated times (in seconds) with percentile ranges of time spent in *Social Investigation* in *isolated* animals treated with 25 mg/kg of cocaine plus glutamate and dopamine antagonists. Differs on two-tailed Mann–Whitney *U*-test from Saline+Saline group ** $p<0.02$; *** $p<0.001$; and from Saline+Cocaine group τ $p<0.02$; τττ $p<0.001$.

comparison with controls. Conversely, animals treated with cocaine and the high dose of SCH spent less time in this behaviour ($p<0.001$). Groups treated with cocaine plus the high dose of CGS ($p<0.02$) or any raclopride dose ($p<0.02$ for the low and $p<0.001$ for the high dose) presented an increase in this behaviour with respect to those receiving only cocaine (see Figs. 1–3).

With regards to *Social Investigation* (Kruskal–Wallis $p<0.001$), interest in conspecifics was reduced after cocaine administration ($p<0.02$). The decrease in these behaviours was also appreciable in the groups treated with cocaine and the low dose of memantine ($p<0.001$), the high dose of CNQX ($p<0.02$), and any of the CGS ($p<0.02$ for the low and $p<0.001$ for the high dose), or raclopride doses ($p<0.001$for the low and $p<0.02$ for the high dose). Animals receiving cocaine plus the low memantine dose ($p<0.02$) or the high CGS dose ($p<0.01$) presented a more profound decrease in social contacts than those receiving only cocaine.

No aggressive behaviours were observed in any of the treatment groups.

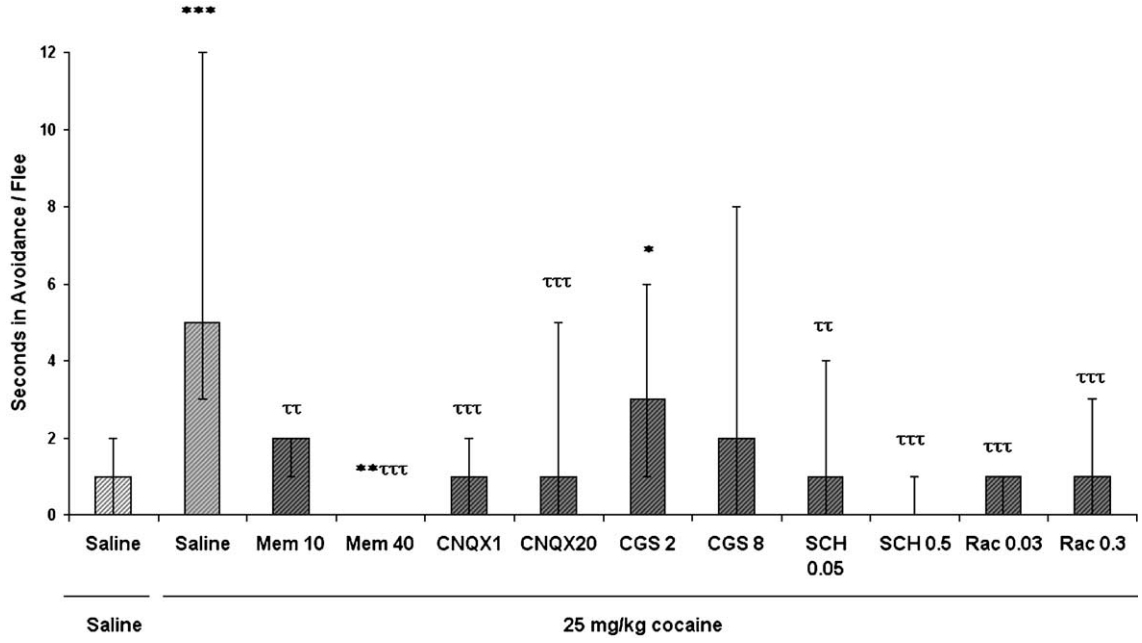

Fig. 6. Medians of accumulated times (in seconds) with percentile ranges of time spent in *Avoidance and Flee* in *isolated* animals treated with 25 mg/kg of cocaine plus glutamate and dopamine antagonists. Differs on two-tailed Mann–Whitney *U*-test from Saline+Saline group * $p<0.05$; ** $p<0.02$; *** $p<0.001$; and from Saline+Cocaine group ττ $p<0.01$; τττ $p<0.001$.

Avoidance/Flee behaviours (Kruskal–Wallis $p<0.001$) were observed (differences with respect to control group) in animals treated with cocaine ($p<0.001$), and cocaine plus the low dose of memantine ($p<0.02$), the low dose of CNQX ($p<0.02$), CGS ($p<0.02$ for both doses), and the low dose of raclopride ($p<0.001$). The cocaine-induced increase in these behaviours was not observed (differences with respect to C25 group) in animals treated with cocaine plus the high memantine dose ($p<0.001$), the high CNQX dose ($p<0.02$), SCH ($p<0.001$ for both doses) and raclopride ($p<0.02$ for the low and $p<0.001$ for the high dose).

3.2. Effect of dopamine and glutamate antagonists on cocaine-induced changes in isolated animals

In Non Social Exploration (Kruskal–Wallis $p<0.001$) all the animals receiving cocaine presented a significant increase in this behaviour with respect to controls ($p<0.001$ for all the groups, except Coc-CGS2 $p<0.05$, Coc-CGS8 and Coc-SCH 0.05 $p<0.01$), apart from those receiving the highest dose of SCH 23390. The two groups receiving SCH 23390 plus cocaine presented a significant decrease in this behaviour with respect to those animals receiving only cocaine ($p<0.001$), but on the contrary, Coc-Mem10 group presented a significant increase with respect to the cocaine group (Coc-Saline) ($p<0.02$) (see Figs. 4–6).

Time spent in Social Investigation (Kruskal–Wallis $p<0.001$) decreased in all the groups injected with cocaine alone or plus memantine, CNQX, raclopride, the high CGS dose and the low SCH dose ($p<0.001$ for all groups except Coc-Rac 0.3 $p<0.02$). The decrease in social contacts induced by cocaine was reversed by the co-administration of any of the SCH 23390 doses ($p<0.001$), and the high raclopride dose ($p<0.02$).

Threat (Kruskal–Wallis $p<0.001$) was abolished after administration of cocaine ($p<0.02$) or cocaine plus memantine ($p<0.001$), CNQX ($p<0.001$), SCH 23390 ($p<0.05$ and $p<0.02$ for the low and high dose, respectively), raclopride ($p<0.001$), and the highest CGS dose ($p<0.05$). Since only saline-treated mice exhibited threat behaviours (mean 32 s) the results for this behaviour are not presented.

Results found in *Attack* (Kruskal–Wallis $p<0.001$) were similar to those found in threat, with an abolition of this behaviour after cocaine ($p<0.001$) or cocaine plus memantine ($p<0.001$), CNQX ($p<0.001$), CGS ($p<0.001$ and $p<0.05$ for the low and high dose, respectively), SCH 23390 ($p<0.001$ and $p<0.02$ for the low and high dose, respectively), and raclopride ($p<0.001$). Similar to the previous behaviour, only saline-treated mice exhibited attack behaviours (mean 45 s) and neither are the results for this behaviour presented.

Avoidance/Flee behaviours (Kruskal–Wallis $p<0.001$) were observed in animals treated with cocaine ($p<0.001$), and cocaine plus the low dose of CGS ($p<0.05$). Conversely, a significant decrease with respect to control animals was observed in the group receiving cocaine plus 40 mg/kg of memantine ($p<0.02$). The cocaine-induced increase in these behaviours was not found in animals treated with cocaine plus any dose of memantine, CNQX, SCH and raclopride ($p<0.001$ in all cases, except $p<0.01$ for the lowest memantine and SCH doses).

4. Discussion

Our results show that both DA and glutamate receptors are implicated in the effect of cocaine on social behaviour. To our knowledge, this is the first work evaluating the relative role of both neurotransmitter systems in the social actions of cocaine, although their influence on other effects of cocaine, such as reward, has been proved. Within the glutamate receptors, NMDA seems to play a more important role, as memantine administration reverts most of the cocaine effects, AMPA receptors being more involved in the increase observed in avoidance and flee behaviours. The D1 DA receptor appears to be more important for cocaine effects than the D2, which is only effective reverting the cocaine-induced increases in avoidance and flee behaviours.

As we have previously reported (Estelles et al., 2004) in grouped or isolated animals, cocaine administration reduces the interest for conspecifics, shown in a decrease in time spent in Social Investigation and the mean duration of encounters (Unit of Social Investigation), and induces an increase in avoidance and flee behaviours. Only in isolated animals does cocaine present a clear hyperlocomotion effect with an increase in time spent in Non Social Exploration and an efficient abolishment of the characteristic increase in all aggressive parameters, such as threat or attack. It has to be taken into consideration that stress can affect the locomotor activating effects of cocaine (Maldonado and Kirstein, 2005), and the confrontation with a conspecific has proved to enhance this effect in rats (Marrow et al., 1999). Since an aggressive response was only observed in isolated animals, this could influence the higher locomotor response to cocaine found only in isolated mice. As a whole, cocaine induces a withdrawal from social contacts and increases avoidance behaviours in both types of animals, it being antiaggressive in those with an aggressive profile.

The cocaine-induced increase in DA release produces a cascade of events that includes a transient increase in pre- and post-synaptic glutamate transmission (Kalivas, 2004). The acute and chronic administration of cocaine in rats significantly elevates glutamate levels in various limbic brain areas (Bell et al., 2000). The results found in the present work give support for the glutamate role in the acute effects that this psychostimulant exhibits on social behaviours.

The blockade of NMDA glutamate receptors with memantine affects the majority of cocaine actions. One of the most consistent effects of cocaine is a reduction in the time employed by the animals in social relation with their conspecifics. Memantine reverses this action, but only with the high dose in grouped animals, it being completely ineffective in isolated mice. Moreover, this NMDA antagonist does not induce a significant increase in social behaviour with respect to cocaine-treated animals, which weakens the efficacy of this drug in restoring social components. On the other hand, memantine completely blocks the increase in avoidance and flee behaviours in both isolated and grouped animals. The

NMDA antagonist does not affect the antiaggressive effects of cocaine. Memantine alone presents an antiaggressive effect on isolated mice only with the highest (40 mg/kg) dose tested (Rodriguez-Arias et al., 2002). Thus, with two drugs that act in the same way, a cumulative effect could occur. Similarly, the low memantine dose increases the social withdrawal and the time spend in Non Social Investigation induced by cocaine, thus presenting a potentiating effect. In humans, memantine increases the subjective effects of cocaine in self-report measures (Cornish and Kalivas, 2000) and similar results have been found in different rodent models with other NMDA antagonists such as MK-801 (Ranaldi et al., 1996).

These results suggest that NMDA receptors seem to play a role in the cocaine-induced increase in avoidance and flee behaviours, since memantine blocks this cocaine action irrespective of the housing conditions of the animals. On the contrary, the social withdrawal induced by cocaine involves NMDA receptors depending on the animal's housing condition. A review of the literature suggests that the interactions of NMDA antagonists and psychostimulants are complex. There are previous reports informing that memantine counteracts different cocaine effects, such as convulsions, lethality (Brackett et al., 2000), or conditioned motor activity (Bespalov et al., 2000). The role of NMDA receptors in the reinforcing effects of cocaine is controversial, since on the one hand, memantine prevents the acquisition and expression of CPP induced by cocaine (Kotlinska and Biala, 2000) but on the other hand, although this drug efficiently decreases self-administration (Hyytiä et al., 1999), in a recent report it did not prevent its acquisition in mice (Blokhina et al., 2005). In the light of our results, although memantine blocks the panic-like effects of cocaine, reducing the increase in avoidance and flee behaviours, the inconsistencies found in reverting social withdrawal, and more importantly the potentiating action observed with low doses, may prevent it from becoming a useful tool.

The same as memantine, CNQX completely reverts the cocaine-induced increase in avoidance and flee behaviours. On the other hand, the AMPA antagonist exerts an inconsistent effect on the decrease in social contacts induced by cocaine, being partially effective only in grouped animals, and only with the lower dose employed. Neither are the antiaggressive actions of cocaine affected after CNQX injection in isolated mice. At the doses tested, this drug does not present any effect on aggression (data not shown). Only a small increase in locomotor actions of cocaine, i.e. an increase in Non Social Exploration, were observed after administration of this AMPA antagonist in grouped animals. The fact that CNQX is not only an AMPA antagonists but also acts at the site of the glycine NMDA receptors (Mead and Stephens, 1999), could explain this enhancing effect already shown with memantine. Although drugs acting on AMPA receptors have been less studied, these receptors seem to play a role in some cocaine effects. The current literature suggests that AMPA receptors mediate the expression of cocaine-induced CPP but seem to show a more inconsistent profile when tested in induction (Mead and Stephens, 1999). Inhibition of AMPA glutamate receptors in the N Acc prevents the expression of sensitisation to cocaine (Pierce et al., 1996; Reid and Berger, 1996). Additionally, CNQX infused in this nucleus blocked cocaine-induced increases in extracellular DA (Pap and Bradberry, 1995). Our results suggest that AMPA receptors are not involved in the social withdrawal or antiaggressive actions of cocaine, on the contrary they are necessary for the increases observed in avoidance and flee behaviours. Thus, glutamate receptors seem to play a critical role in the cocaine-induced increase in avoidance and flee behaviours.

Among brain neurotransmitters, DA is the one that is the most extensively implicated in the mechanism of psychostimulant actions (Di Chiara et al., 2004). In mice, cocaine administration significantly increases mean DA levels in the Nucleus Caudate–Putamen and the N Acc (Witkin et al., 2003). Surprisingly, the DA release inhibitor CGS 10746B does not greatly affect behavioural cocaine actions. Neither are the decrease in social contacts or the increase in avoidance and flee behaviour reverted after this drug administration. The antiaggressive effects of cocaine are equally preserved, with both of the doses tested, the high dose alone achieving an antiaggressive effect (Felip et al., 2001). Although the increase in functional levels of DA following cocaine administration is mediated by cocaine's interference with the DA transporter (Ritz et al., 1987), previous reports show that CGS 10746B sensitive-release of DA may be necessary for several actions of cocaine. The DA release inhibitor blocks cocaine-induced place preference (Bilsky et al., 1998), and decreases cocaine discrimination (Schechter, 1993), and cocaine-induced hyperactivity (Calcagnetti and Schechter, 1992; French and Witkin, 1993). Nevertheless, the present results show that dopamine release does not play a pivotal role in social effects of cocaine. The use of low doses may account for the lack of effects observed, since in the studies mentioned previously higher doses were used (20 to 40 mg/kg in rats). Although the high dose of CGS 10746B employed has been proved to be antiaggressive with the same strain of mice (Felip et al., 2001) it may not induce a sufficient effect on DA release to affect cocaine actions.

The blockade of D1 DA receptors with SCH 23390 completely restores the decrease in time dedicated to social contacts, this effect being stronger in isolated mice with a significant increase with respect to cocaine-treated mice. Moreover, after SCH 23390 administration no avoidance or flee behaviours are observed in animals treated with cocaine. Although the high dose of the D1 DA antagonist decreases time dedicated to Non Social Exploration, this effect is not specific, since it is accompanied by an increase in immobility (data not shown). No affectation of the antiaggressive actions of cocaine was observed in isolated animals. At the doses employed, SCH 23390 efficiently decreases aggression in isolation- (Rodriguez-Arias et al., 1998) and morphine-withdrawal-induced aggression (Rodriguez-Arias et al., 1999). In the light of the results, the D1 DA receptors seem to play an essential role for the cocaine effects on social behaviours, as well as in the appearance of avoidance and flee elements. An increase in defensive upright, sideways

postures and escape when confronted with drug-free conspecifics has been previously described after chronic cocaine treatment, although the selective D1 receptor antagonist SCH 23390 did not significantly affect these defensive patterns (Filibeck et al., 1988). These results are not in agreement with our findings, since SCH 23390 fully blocked avoidance and flee.

On the other hand, our results are in accordance with other reports in which SCH 23390 blocked many of cocaine's actions. Systemic administration of SCH 23390 reversed cocaine-induced locomotion, sniffing and lethality (Baker et al., 1998; Schechter and Meehan, 1995). Its effect on cocaine-induced CPP is controversial: while some authors found that this D1 antagonist blocked this preference (Sanchez et al., 2003), others found no effect (Shippenberg and Heidbreder, 1995; Liao et al., 1998). Moreover, the rate of cocaine self-administration is increased after SCH 23390 (Caine et al., 1995; Phillips et al., 1994). Nevertheless, an agreement exists that SCH 23390 is capable of blocking the reinstatement of cocaine, either in the CPP or self-administration procedure (Sanchez et al., 2003; Sun and Rebec, 2005). Furthermore, conditioned reinstatement of drug-seeking behaviour is blocked by SCH 23390 when injected in the amygdala (See et al., 2001).

The blockade of D2 DA receptors does not restore the social contacts of cocaine-treated mice, but on the contrary, raclopride completely counteracts the cocaine-induced increase in avoidance and flee behaviours. No affectation of antiaggressive or motor effect of cocaine is appreciated after D2 DA antagonist injection. The high raclopride dose exerts an antiaggressive action on both isolation- (Aguilar et al., 1994) and morphine-withdrawal-induced aggression (Rodriguez-Arias et al., 1999). These results suggest that D2 DA receptors would be only involved in cocaine-induced avoidance and flee, these results being in concordance with the finding that the selective D2 receptor antagonist (−)-sulpiride (25 mg/kg) administered before the challenge dose of cocaine completely antagonized the increase in defensive behaviour (Filibeck et al., 1988).

The role of D2 DA receptors has not been demonstrated for all of cocaine's actions. Knockout mice for these receptors showed reduced levels of horizontal activity relative to wild mice after cocaine administration, although mutant mice are capable of acquiring the discrimination of cocaine (Chausmer et al., 2002). Raclopride did not affect either cocaine-induced CPP or the development of sensitisation (Shippenberg and Heidbreder, 1995), although other D2 DA receptor blockers, such as eticlopride, increased cocaine self-administration and decreased cocaine-primed reinstatement (Sun and Rebec, 2005).

Our results have shown that both D1 and D2 receptor antagonists block the increase observed in avoidance and flee behaviours after cocaine administration. Conversely, Filibeck et al. (1988) found that a challenge dose of cocaine after the end of chronic intermittent treatment with the psychostimulant induced an increase in defensive upright and sideways postures that were completely antagonized only with the D2 receptor antagonist (−)-sulpiride, while the selective D1 receptor antagonist SCH 23390 did not significantly affect these defence patterns. The different schedule of cocaine treatment (chronic vs. acute), the specific behaviours evaluated, the different strain of mice used and the fact that raclopride also acts as an antagonist of the D3 receptor, could explain this lack of concordance.

There is considerable evidence that environmental manipulations such as variations in the social housing conditions of the animal may play a role in the expression of individual differences in response to drugs. Isolated and grouped animals present dramatic behavioural differences, the former being more aggressive and active than the latter. The study of isolated and grouped animals allows us to reveal the changes in dopamine or glutamate receptors that these housing conditions could induce. Isolation rearing might interact with dopamine-dependent mechanisms (Jones et al., 1990), which could lead to diverse responses to cocaine (Estelles et al., 2005) or morphine (Broseta et al., 2005). In the present work, differences depending on the housing conditions of the animals have been particularly observed after memantine administration, which is capable of restoring time dedicated to social investigation only in grouped animals. No difference in the action of the DA antagonists employed has been observed, although the role of these receptors seems to be affected by rearing conditions when the cocaine-induced reinforcing effects are studied. A divergence in the effect of intraaccumbens infusion of SCH23390 and sulpiride has been described, socially reared rats being more responsive than isolated rats in enhancing the rate of cocaine self-administration (Phillips et al., 1994).

Previous reports have shown that different dopamine and glutamate receptors present diverse effects on cocaine actions. For instance, using the place preference paradigm the D1 DA, but not the D2, and the glutamatergic NMDA receptors are involved in the primary rewarding properties of cocaine, whereas AMPA receptors are only important for the behaviour elicited by stimuli previously associated with the drug action (CPP expression) (Cervo and Samanin, 1995). In the present work cocaine-induced avoidance and flee elements is abolished by both glutamate and dopamine receptors. On the contrary, social withdrawal has been less sensitive to the blockade of these receptors, the DA D1 being the more relevant. Social behaviours are difficult to evaluate using only this model, since anxiety, aggression and social interest could be hard to separate. Other studies involving a specific model of anxiety, such as the plus maze, would help to separate these components.

The increase in defensive elements and the reduction or abolishment of social contacts induced by cocaine administration suggests an axiogenic-like action. The drugs tested in the present work could effectively deal with the problems associated with cocaine use and the present data could help in the particularly difficult treatment of cocaine abusers. Although both neurotransmitter systems are involved in cocaine effects, in the light of our results, NMDA and D1 DA receptors seem to have an important role.

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