Psychopharmacology (2004) 171:148–155
DOI 10.1007/s00213-003-1578-8

ORIGINAL INVESTIGATION

Alberto Del Arco · Shunwei Zhu · Anton Terasmaa · Abdul H. Mohammed · Kjell Fuxe

Hyperactivity to novelty induced by social isolation is not correlated with changes in D2 receptor function and binding in striatum

Received: 3 November 2002 / Accepted: 18 June 2003 / Published online: 10 September 2003

Abstract *Rationale:* Prolonged social isolation has been reported to induce different behavioral disturbances, among the most consistent of which are the increased locomotor response to novelty and the effects of psychostimulants. While these behavioral changes have been partly related to a dysregulation of dopaminergic activity in striatum (dorsal and ventral), the involvement of changes in the function of dopamine receptors is still a matter of controversy. *Objectives:* To investigate the effects of prolonged social isolation on the function of D2 receptors at both the behavioral and biochemical levels. *Methods:* Sprague-Dawley rats were randomly placed at 21 days of age in groups or isolation for 2 months. Horizontal and vertical locomotor activities induced by novelty and also by systemic injections of the D2 agonist quinpirole (0.15, 0.50 and 1.5 mg/kg i.p.) and their modulation by the A2A agonist CGS 21680 (0.1 mg/kg i.p.) were studied. The effects of social isolation on the avoidance learning assessed by the passive avoidance test were also studied. Binding experiments were performed to study the number and affinity of D2 receptors by means of saturation and competition experiments with the D2 antagonist [^{3}H]-raclopride and the interaction between D2 receptors and the G-protein by means of [^{35}S]-GTPγs binding in dorsal/ventral striatal membranes of both grouped and isolated rats. *Results:* Rats reared in isolation were hyperactive to a novel environment and showed shorter retention latencies in the passive avoidance test. Isolation rearing did not modify the increase in motor activity produced by quinpirole nor the counteraction of these effects by the simultaneous stimulation of A2A receptors. Likewise, the number, affinity and functional efficacy of D2 receptors were not changed by social isolation. *Conclusions:* These results suggest that the hyperactivity to novelty and psychostimulants as well as other behavioral changes induced by social isolation do not parallel changes in the in vivo function or binding of D2 receptors in dorsal/ventral striatum.

Keywords Isolation rearing · Dopamine · D2 receptors · A2A receptors · Motor activity · Passive avoidance · Striatum · Rat

A. Del Arco · A. Terasmaa · K. Fuxe
Department of Neuroscience,
Karolinska Institutet,
171 77 Stockholm, Sweden

A. Del Arco (✉)
Department of Physiology, Faculty of Medicine,
Universidad Complutense, Ciudad Universitaria,
s/n, 28040 Madrid, Spain
e-mail: Alberto.Del.Arco@neuro.ki.se
Fax: +34-91-3941628

S. Zhu · A. H. Mohammed
Division of Geriatric Medicine, NEUROTEC,
Karolinska Institutet, Huddinge University Hospital,
Sweden

Introduction

Social isolation has been shown to induce a syndrome of different neurochemical and behavioral changes (Robbins et al. 1996; Heidbreder et al. 2000), some of which seem to resemble those found in schizophrenic-like psychosis (Robbins et al. 1996; Weiss and Feldon 2001). For instance, rats reared in isolation show increased locomotor activity in response to novel environments (Gentsch et al. 1982; Hall et al. 1998; Heidbreder et al. 2000), are more sensitive to the effects of psychostimulant drugs such as amphetamine (Jones et al. 1990, 1992; Hall et al. 1998) and cocaine (Howes et al. 2000), and present impairments in sensorimotor gating processes as assessed in the prepulse inhibition paradigm (Varty et al. 2000; Heidbreder et al. 2000; Weiss and Feldon 2001). Many of these behavioral changes induced by social isolation have been related to changes in the function of the mesolimbic dopaminergic system (Robbins et al. 1996). In particular, the behavioral hyper-reactivity to arousal stimuli induced by social isolation has been related to an increase in the release of dopamine in the dorsal and ventral striatum. In fact, several studies have reported that the pharmacological and behavioral stimulation of the dopaminergic

system by amphetamine/cocaine injections and stress, respectively, produce higher increases of dopamine in the dorsal and ventral striatum of rats reared in isolation relative to grouped rats (Jones et al. 1992; Fulford and Marsden 1998; Hall et al. 1998; Howes et al. 2000).

However, and since spontaneously hyperactive rats have been shown to have changes in the density and expression of dopamine D1 and D2 receptors in the dorsal and ventral striatum (Hooks et al. 1994b), changes at the dopamine receptor level in these areas of the brain of rats reared in isolation might be expected. D2 receptors become particularly relevant in this context since a close relationship has been suggested between striatal D2 receptor changes and behavioral dysfunctions in psychiatric disorders such as schizophrenia (Abi-Dargham et al. 2000; Seeman and Kapur 2000). However, the existing and limited information available on the effects of social isolation on D2 receptor function in the brain is still unclear. For instance, at the behavioral level, a reduced sensitivity has been shown to systemic or intracerebral injections into the nucleus accumbens of D2 antagonists in isolated rats, which suggests a functional downregulation of D2 receptors in these rats (Phillips et al. 1994; Sundstrom et al. 2002). However, other studies have shown that the effects of intra-perifornical injections of D2 antagonists stimulating motor activity are enhanced in isolated as opposed to grouped rats (Morutto and Phillips 1997). There are no reports investigating the effects of the specific activation of D2 receptors in isolated rats. At the receptor level, the density of D2 receptors has been reported to be increased (Guisado et al. 1980) in the dorsal striatum or unchanged (Bardo and Hammer 1991) in the dorsal and ventral striatum of rats reared in isolation. Using a more functional approach, the inhibition of cAMP formation by D2 receptor stimulation has been shown to be reduced (Hall et al. 1998) in the ventral striatum (nucleus accumbens) and unchanged (Jones et al. 1992) in the dorsal striatum of isolates.

The aim of the present study was to investigate whether D2 receptor function, at both the behavioral and biochemical levels, was modified in rats reared in isolation. We investigated: first, the effects of social isolation in two behavioral tests, novelty-induced motor activity in the open field and avoidance learning using the passive avoidance paradigm; second, the effects of social isolation on the increase in motor activity induced by the D2-like agonist quinpirole and the counteraction of these effects by the A2A agonist CGS 21680 [A2A-D2 interaction (Ferre et al. 1997)]; and third, in a series of binding experiments, we investigated whether social isolation had any effect on the density and affinity of D2 receptor binding sites (by means of saturation experiments with the D2 antagonist [^{3}H]-raclopride and competition experiments with [^{3}H]-raclopride versus dopamine) or on the efficacy and potency of dopamine at D2 receptors in dorsal/ventral striatal membranes (by studying the ability of dopamine to stimulate the G-protein coupling as analyzed with the [^{35}S]-GTPγS binding assay).

Methods

Animals and housing conditions

The present study was conducted on Sprague Dawley male rats (Alab, Sweden). The animals arrived at the facility just at weaning (21 days old) and were randomly placed in two different housing conditions: individually (isolated) in standard plexiglas cages (40×25×15 cm) or in groups of four (grouped), also in plexiglas cages (55×35×20 cm). Both groups of animals were housed under the same conditions in temperature-controlled rooms (21°C), with a 12-h/12-h light/dark cycle (lights on/off at 0700/1900 hours) and provided with food and water ad libitum. The animals remained in these conditions for at least 8 weeks prior to testing and thereafter for the duration of the experiments. During this time, the animals received minimal handling during cage cleaning twice a week. Isolated rats could see, smell and hear other rats so that only physical interaction was prevented. Experiments were carried out in accordance with the regulation of The Swedish National Board for Laboratory Animals (CNF, Dnr. S49/01).

Behavioral tests

Motor activity

Novelty- and pharmacologically induced motor activity were carried out in four automated open field arenas. The open field apparatus consisted of a plexiglas box (70×70×45 cm) equipped with two horizontal rows of eight infrared light-sensitive photo beams located at 5 cm and 15 cm, respectively, from the basement allowing the detection of the location of the rat within the arena. Interruptions of the photocell beams were registered automatically by a computer. The following parameters were recorded for each animal: central and peripheral locomotion (horizontal activity) and central and peripheral rearing (vertical activity). Open field activities were recorded every 10 min during a period of 60 min to measure novelty-induced motor activity (this was the first time rats were exposed to the open field), and for 120 min plus 30 min habituation period to measure quinpirole-induced motor activity. Two isolated rats and two grouped rats were always tested simultaneously in the four arenas. (–) Quinpirole hydrochloride (Tocris Cookson Ltd) (0.15, 0.50 and 1.50 mg/kg) and CGS21680 (RBI) (0.10 mg/kg.) were dissolved in 0.9% saline for intraperitoneal injections. Open field tests were carried out between 1200 hours and 1600 hours. The arena was wiped with 70% ethanol followed by water between rats.

Passive avoidance

Five to nine days after open field tests, grouped and isolated rats were trained and tested in a passive avoidance apparatus consisting of a shuttle-box divided into two compartments, separated by a sliding door. The starting compartment (light compartment) (45×45×19 cm) was made of white opaque plastic and was well lit; the shock compartment (dark compartment) (25×24×19 cm) was made of black plastic and equipped with a removable cover to provide darkness and with a electrifiable grid floor to deliver a foot shock. The passive avoidance test was carried out in 2 days. The training day consisted of two sessions: (1) rats were placed in the starting compartment (light compartment) and were allowed to explore the whole apparatus (sliding door open) over a period of 300 s. The time to enter the dark compartment (training latency) and the time spent in both compartments were recorded; (2) 2–3 h later, rats were returned to the apparatus and placed in the light compartment. When they stepped with all four paws onto the dark side, the sliding door was closed and a single foot shock (0.5 mA, 2 s) was delivered. After 10 s, animals were removed from the dark compartment and returned to their home cages. On the test day, 24 h later, animals were placed in the light compartment and, as in the training session, the time to enter the dark compartment

(retention latency) with all four paws and the time spent in both compartments were recorded for 600 s (cut off). Shock was not administered at the retention test trial.

Dopamine radioligand ([^{3}H]-raclopride) binding assay procedure

Rats were sacrificed by decapitation, the brains were rapidly removed and the whole striatum (dorsal and ventral, right and left) was dissected out on a cooled glass plate, immediately frozen in liquid nitrogen and stored at −80°C pending analysis. Tissue pieces were placed in vials with 10 ml ice-cold pre-incubation buffer (20 mM Tris-HCl, 5 mM $MgCl_2$ and 1 mM EDTA, pH 7.4) and homogenized by sonication. The membranes were collected by centrifugation at 20,000 rpm for 20 min at 4°C and the supernatant was discarded. The pellet was re-suspended in the same buffer and centrifuged again as above. The final pellet was homogenized into incubation buffer (20 mM Tris-HCl, 5 mM $MgCl_2$, 1 mM EDTA, 100 mM NaCl, and 1 mM DL-dithiothreitol, pH 7.4) at a tissue concentration of 3 mg/ml. Protein content was measured by the modified method of Lowry (Peterson 1983) using bovine serum albumin as a standard.

Binding of the D2 receptor antagonist [metoxy-^{3}H]-raclopride (76 Ci/mmol) (NEN Life Science Products, Boston, MA, USA) to striatal membranes was performed as described elsewhere (Terasmaa et al. 2000). The reaction was started by addition of 250 µl of membranes to incubation buffer containing different concentrations of [^{3}H]-raclopride (final 0.2–10 nM) for 90 min at 25°C in a total volume of 400 µl. Non-specific binding was defined as the binding in presence of (+)butaclamol 10 µM (Sigma, Sweden). Competition experiments with dopamine versus [^{3}H]-raclopride were performed with 20 concentrations (1nM to 1 mM) of dopamine (Sigma, Sweden) and [^{3}H]-raclopride (2 nM) and with/without 10 µM GTPγS (Sigma, Sweden) following the same general procedure as in the saturation experiments.

Binding of the [^{35}S]-GTPγS (1250 Ci/mmol) (NEN Life Science Products, Boston, MA, USA) versus dopamine to striatal membranes (Terasmaa et al. 2000) was performed with 0.05 nM [^{35}S]-GTPγS and 12 concentrations of dopamine (1nM to 1 mM) and GDP 300 µM (Sigma, Sweden) following the same general procedure as in the competition experiments with [^{3}H]-raclopride described above.

In each binding experiment, brain tissues from isolated and grouped rats were assayed simultaneously. The binding reaction was terminated by addition of ice-cold buffer (20 mM Tris-HCl and 100 mM NaCl, pH 7.4) and immediately separated by rapid filtration through wet glass-fiber filters (GF/B, Whatman Int. Ltd., Maidstone, UK) and the filters were washed two times with 5 ml ice-cold buffer. Filters were placed in scintillation vials together with 5 ml scintillation cocktail (Flo-Scint V, Packard Bioscience B.V.). Vials were allowed to set overnight, and radioactivity was counted by means of a scintillation spectrometer. All binding data were analyzed by means of the non-linear least-squares regression method using the GraphPad PRISM software.

Statistical analysis

Two-way analysis of variance (ANOVA) with repeated measures was performed considering time and rearing condition as within- and between-subject factors, respectively, followed by post-hoc comparisons to analyze motor activity induced by novelty. Total motor activity counts during 110 min produced by quinpirole injections, alone or in combination with CGS21680, were analyzed by a two-way ANOVA (drug treatment × rearing condition). The analysis of the passive avoidance data (retention latencies, time spent in light and dark compartments) was performed using the Kruskal-Wallis test by ranks as the latencies of some rats were above the cut off. Binding parameters were analyzed using paired or unpaired Student's *t*-tests.

Results

Effects of social isolation on novelty-induced motor activity and avoidance learning

As shown in Fig. 1, novelty-induced horizontal motor activity was significantly higher in rats reared in isolation than in grouped rats [$F_{1,46}$=4.46, P<0.04, n=48 (24 per group)]. The temporal profile of this effect indicates that isolated rats were significantly hyperactive during the first 20 min (T10: $F_{1,46}$=16.04, P<0.0002 and T20: $F_{1,46}$=4.54, P<0.03). Social isolation did not significantly modify vertical motor activity ($F_{1,46}$=1.02, P<0.32).

As shown in Fig. 2, isolated rats showed significantly lower retention latencies than grouped rats 24 h after training, but not on the training day (see insert), in the passive avoidance test [Kruskal-Wallis by ranks $H_{1,34}$=7.07, P<0.0078, n=34 (17 per group)].

Time out of 600 s (cut off) that both groups of rats spent in the light and dark (footshock) compartments on the test day was also measured. Rats reared in isolation spent shorter time in the light [Kruskal-Wallis by ranks $H_{1,34}$=10.13, P<0.0015] and longer time in the dark [Kruskal-Wallis by ranks $H_{1,34}$=6.76, P<0.009] than grouped rats.

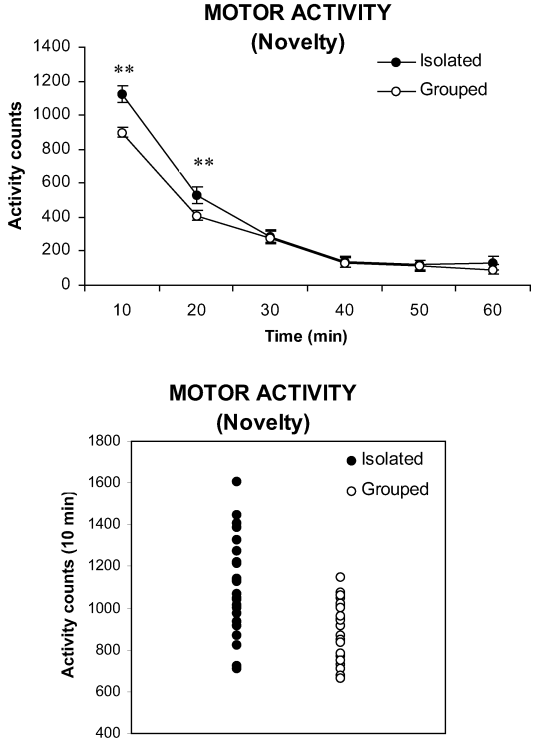

Fig. 1 Effects of social isolation on the motor activity (horizontal activity) to novelty (first day of exposure) in the open field. *Top* Temporal profile of the effects of social isolation on motor activity. The data are presented as the mean±SEM. **P<0.03 after post-hoc comparisons (n=48, 24 per group). *Bottom* Activity counts of isolated relative to grouped rats during the first 10 min in the novel environment. Every *circle* represents one animal

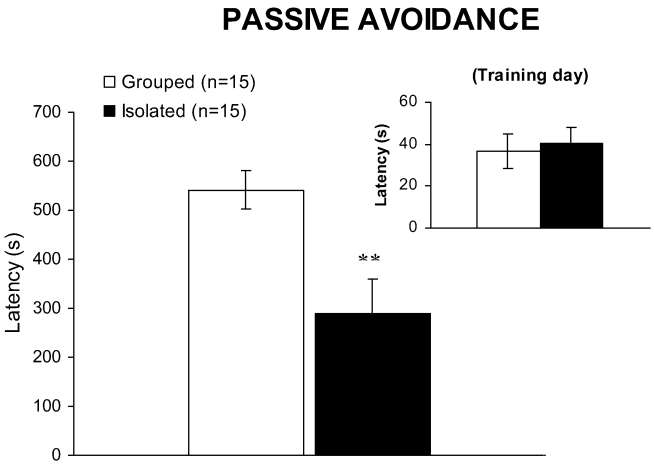

Fig. 2 Effects of social isolation on the retention latency 24 h after training in the passive avoidance behavioral test. The *insert* shows the retention latency on the training day prior to the footshock. *Bars* represent the mean±SEM. $^{**}P<0.01$ relative to grouped using the Kruskal-wallis test by ranks (n=34, 17 per group)

Effects of social isolation on quinpirole-induced motor activity: counteraction by the A2A agonist CGS 21680

The D2-like receptor agonist quinpirole (0.15, 0.50 and 1.50 mg/kg i.p.) was used to investigate whether the behavioral effects produced by specific stimulation of D2 receptors were affected by social isolation. As shown in Fig. 3 quinpirole (0.15, 0.50 and 1.50 mg/kg i.p.) significantly increased horizontal motor activity in isolated and grouped rats relative to saline treatment ($F_{3,38}$=9.91, P<0.00006, n=5–7) but these effects were not modified by rearing conditions ($F_{1,38}$=0.29, P<0.59). Quinpirole (0.15, 0.50 and 1.50 mg/kg i.p.) also increased vertical motor activity in isolated and grouped rats, although these effects did not reach statistical significance due to the high variability within groups ($F_{3,38}$=2.62, P<0.06, n=5–7), and these effects were not modified by rearing conditions (ANOVA $F_{1,38}$=0.009, P<0.9) (Fig. 3).

The A2A agonist CGS 21680 (0.10 mg/kg i.p.) together with quinpirole (0.15 mg/kg i.p.) was used to investigate whether the behavioral effects produced by the A2A–D2 receptor interaction (Ferre et al. 1997) were affected by social isolation. As shown in Fig. 4, the activation of A2A receptors significantly attenuated the increases in motor activity produced by quinpirole in isolated ($F_{1,21}$=22.2, P<0.0001, n=6) as well as in grouped ($F_{1,21}$=21.6, P<0.0001, n=6–7) rats. Neither the effects of quinpirole alone nor the effects of quinpirole + CGS 21680 on motor activity were significantly changed by social isolation ($F_{1,21}$=0.95, P<0.76).

Effects of social isolation on D2 receptor binding and function in dorsal/ventral striatum: biochemical analysis

As shown in Table 1, there were no differences between grouped and isolated rats in the density of specific D2

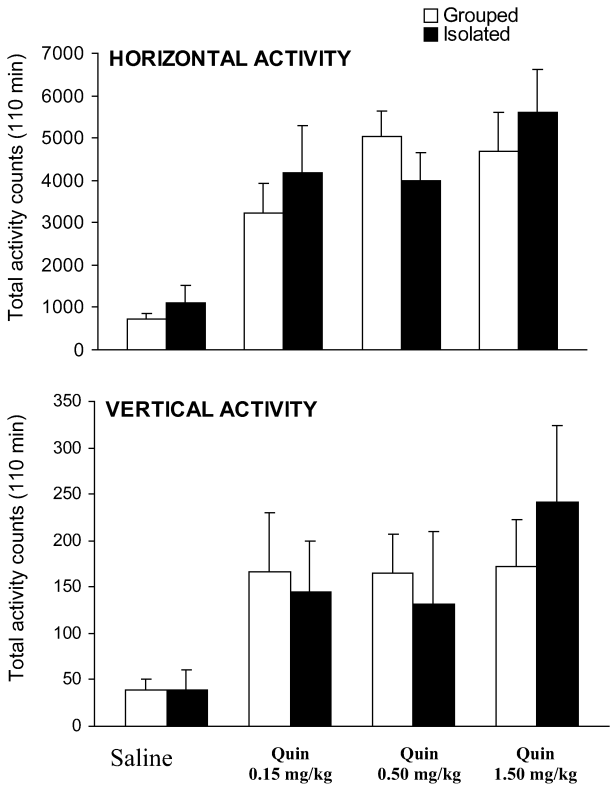

Fig. 3 Effects of social isolation on the motor activity induced by injections of different doses of the D2-like agonist quinpirole (i.p.) in the open field. *Bars* represent the mean±SEM of the total activity counts recorded during 110 min, starting 10 min after the injection (n=5–7)

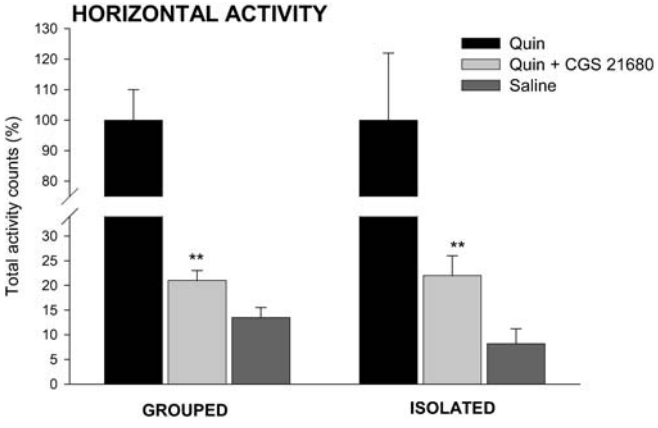

Fig. 4 Effects of social isolation on the effects of the A2A agonist CGS 21680 (0.1 mg/kg, i.p.) on the increases in motor activity (horizontal activity) produced by the D2-like agonist quinpirole (0.15 mg/kg, i.p.) in the open field. *Bars* represent percentages of the mean±SEM of the total activity counts recorded during 110 min. Quin group in isolated and grouped rats is set as 100%. $^{**}P$<0.001 relative to respective quin group using the two-way ANOVA followed by post-hoc procedures (n=6–7)

Table 1 Effects of social isolation on the K_D and B_{max} values of D2 antagonist binding sites in striatal membranes using [^{3}H]-raclopride. B_{max} and K_D values are expressed as means±SEM (n=4–5)

	Grouped	Isolated
B_{max} (fmol/mg protein)	393±58	324±69
K_D (nM)	0.88±0.05	0.85±0.08

Table 2 Effects of social isolation on the K_H, K_L and R_H values of dopamine for D2 receptors based on competition experiments of [3H]-raclopride versus dopamine. K_H and K_L values are expressed as geometric means (antilogarithms of the logarithmically transformed data) and in parenthesis the 95% confidence limits of the geometric mean. R_H values are expressed as means±SEM (n=4–5)

	Grouped	Isolated
K_H (nM)	6.4 (2.2–19.0)	5.9 (1.3–26.8)
K_L (nM)	294 (70–1225)	358 (54–2345)
R_H (%)	55±9%	59±8%

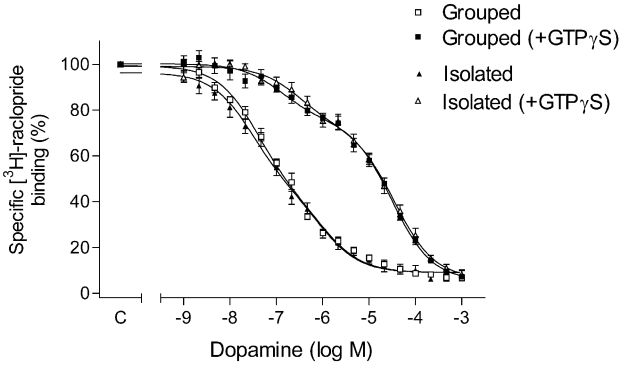

Fig. 5 Effects of social isolation on the displacement of the D2 antagonist [^{3}H]-raclopride by dopamine in dorsal/ventral striatal membrane preparations. The figure represents the competitive curves with dopamine versus [^{3}H]-raclopride from grouped and isolated rats (see binding parameters in Table 2). GTPγS 10 μM induced a similar shift to the right in the competition curves (see text) of both grouped and isolated rats [$K_{H(grouped)}$=42.8 μM (19–94 μM) and $K_{H(isolated)}$=74.5 μM (33–166 μM), t_7=1.51 (n.s.) comparing grouped and isolated K_H values]. The data represent percentages and are shown as mean±SEM of 4–5 independent experiments (C=control, point without dopamine)

antagonist binding sites (B_{max}) (t_6=0.74, P<0.48) nor in their affinity (K_D) (t_8=0.76, P<0.46) in dorsal/ventral striatal membranes. The total protein content in the membranes did not change significantly: 55±6 mg and 57±3 mg protein/g wet tissue for grouped and isolated rats, respectively.

Displacement of [^{3}H]-raclopride binding by dopamine was best described according to a two-site binding model with high (K_H) and low (K_L) affinity constants in both groups of rats. Table 2 and Fig. 5 show that there were no significant differences in K_H (t_8=0.34, P<0.72) and K_L (t_8=0.68, P<0.51) values nor in the proportion of high-affinity binding sites (R_H) (t_8=0.28, P<0.78) when comparing both groups of rats. The addition of 10 μM GTPγS to the incubation medium shifted the displacement curves to the right by decreasing the fraction of high-affinity binding sites from 55±9% to 27±3% and from 59±8% to 28±3% in the grouped and isolated rats, respectively (see Fig. 5).

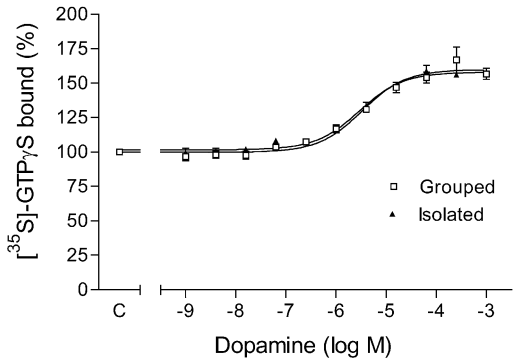

Fig. 6 Effects of social isolation on the dopamine-induced [^{35}S]-GTPγS specific binding in dorsal/ventral striatal membrane preparations. The data represent mean±SEM of three independent experiments and are expressed as percentage stimulation above basal in the grouped and isolated rats (C=control, point without dopamine)

Figure 6 shows the activation of the [^{35}S]GTPγS binding to membranes from dorsal/ventral striatum of grouped and isolated rats produced by dopamine in the presence of GDP 300 μM. The maximal dopamine-stimulated [^{35}S]GTPγS binding was 58±3% and 55±1% above basal values for grouped and isolated rats, respectively, and was not statistically different. Likewise there were not differences in the concentrations of dopamine causing a half-maximal stimulation of [^{35}S]GTPγS binding with EC_{50}=2.75 μM (0.84–4.80 μM) (n=3) for grouped rats and EC_{50}=3.19 μM (0.79–5.83 μM) (n=3) for isolated rats. Basal [^{35}S]GTPγS binding was 9422±2673 d.p.m./tube protein for grouped rats and 10073±4568 d.p.m./tube protein for isolated rats.

Discussion

The main findings of the present study can be summarized as follows.

1. Rats reared in isolation are hyperactive to a novel environment and show impaired retention 24 h after training in the passive avoidance test
2. Social isolation does not modify either the increase in motor activity produced by the D2 agonist quinpirole or the counteracting of these effects by the simultaneous stimulation of A2A receptors
3. Social isolation does not change the number, affinity or functional efficacy and potency of dopamine at D2 receptors in dorsal/ventral striatal membranes.

These results suggest that the hyperactivity to novel environment or psychostimulants induced by social isolation is not correlated with changes in the in vivo

function, and binding of D2 receptors in the dorsal/ventral striatum.

Early and prolonged social isolation has been reported to induce behavioral and neurochemical changes (for review Robbins et al. 1996; Heidbreder et al. 2000), among the most consistent of which are the increased locomotor response to novelty and to the effects of amphetamine (Gentsch et al. 1982; Hall et al. 1998; Heidbreder et al. 2000). In the present study and in agreement with the above-mentioned work, rats reared in isolation were hyperactive the first day they were exposed to the open field. Whether this hyperactivity reflects an alteration in exploratory behavior or anxiety, or the preference for a novel environment is still a matter of discussion since the effects of social isolation on spontaneous motor activity seem to depend on a multitude of factors such as the strain, housing conditions and the aversive properties of the testing environment (Holson et al. 1991; Hall et al. 1997, 2000; Heidbreder et al. 2000).

The hyper-reactivity to arousing stimuli induced by social isolation has been related to changes in the function of the dopaminergic mesolimbic system (Robbins et al. 1996). Thus, microdialysis experiments have shown that psychostimulants such as amphetamine and cocaine and also stress produce higher increases of dopamine in the ventral, and also dorsal, striatum (Jones et al. 1992; Fulford and Marsden 1998; Hall et al. 1998; Howes et al. 2000) of rats reared in isolation relative to grouped rats, which suggests presynaptic changes in the release of dopamine induced by social isolation. However, the possibility that social isolation produces postsynaptic changes involving the function of dopamine receptors is still unresolved. In the present study, the first approach to investigate possible postsynaptic changes of D2 receptors in particular was to study whether social isolation changes the effects at the behavioral level of the specific activation of D2 receptors by the D2-like agonist quinpirole. It is of interest in this respect that, according to previous studies, the ventral part of striatum (nucleus accumbens), more than the dorsal part, seems to account for the motor-stimulating effects (locomotion and rearing) produced by dopaminergic drugs (Kelly and Iversen 1976; Kelley et al. 1989). As shown in the results section, intraperitoneal injections of quinpirole in all doses used, produced similar increases in motor activity in both isolated and grouped rats. These results suggest that the in vivo activation of D2 receptors is resistant to rearing conditions and also that changes in D2 receptor function, possibly in the ventral striatum, are not involved in the enhanced increases in locomotor activity in isolated rats induced by novelty and amphetamine. The results obtained in the present study seem to be complementary to previous in vivo studies that, using a different approach (blocking instead of activating D2 receptors), have found changes in D2 sensitivity in isolated relative to grouped rats (Phillips et al. 1994; Morutto and Phillips 1997; Sundstrom et al. 2002) in different areas of the brain, such as nucleus accumbens (Phillips et al. 1997) and lateral hypothalamus (Morutto and Phillips 1997).

The function of D2 receptors can be directly or indirectly modulated by other neurotransmitters and neuromodulators. In particular, the antagonistic interaction between D2 and A2A receptors has been well established at both the behavioral and the molecular level (Ferre et al. 1997). Indeed it has been shown that the stimulation of A2A receptors strongly reduces the increase in motor activity produced by the stimulation of D2 receptors (Strömberg et al. 2000) and this has been suggested to be produced by intra-membrane receptor–receptor interaction since A2A agonists are able to reduce the affinity of D2 receptors for dopamine in striatal membranes (Ferre et al. 1991). Furthermore, the A2A–D2 interaction seems to be predominant in the ventral striatum, suggesting a role for A2A receptors as antipsychotic targets (Ferre 1997). In the present study, we investigated whether the in vivo A2A–D2 interaction, more than D2 itself, was affected by social isolation. As isolated rats were hyperactive, a functional downregulation of A2A receptors in these rats could be expected and thus a weaker ability of A2A agonists to counteract the D2-stimulated motor activity. As shown in the result section, the stimulation of A2A receptors with the specific agonist CGS 21680 equally counteracted the increases in motor activity produced by quinpirole in isolated and grouped rats. These results are consistent with an intact D2 receptor function at the behavioral level in isolated rats and can be extended to suggest that A2A receptors and A2A–D2 interaction, predominantly in the ventral striatum, are not affected by rearing conditions.

The lack of changes observed in the present study regarding the in vivo stimulation of D2 receptors only gives indirect information on possible alterations of D2 function at the receptor level (Hooks et al. 1994a, 1994b). Therefore, biochemical assays were also carried out in this study to investigate D2 function at the receptor level. Since changes in the release of dopamine produced by social isolation have been observed in both dorsal and ventral parts of striatum, changes of D2 receptors may be expected in these regions of the brain. According to this, we studied in the first series of D2 binding experiments in striatal membranes (dorsal/ventral striatum) whether social isolation altered the density and affinity of dopamine for D2 receptors. As shown in Table 1, Table 2 and Fig. 5, there were no changes in the density and affinity of D2 receptors in isolated rats relative to grouped rats as studied with the D2 antagonist radioligand nor in the ability of dopamine to compete with the D2 antagonist radioligand for the D2 receptors as seen from the unchanged K_H, K_L and R_H values. These results are in agreement with other studies showing no changes in the number of D2 receptors either in dorsal or in ventral striatum of rats reared in isolation (Bardo and Hammer 1991) and extend these findings by showing that the affinity of dopamine for the high and low affinity states of the D2 receptors is unaltered by social isolation as is also the proportion of D2 receptors in the high affinity state.

In another series of binding experiments and using the same approach, we studied the functional interaction

between D2 receptors and the G-protein (assessed by means of the GTPγS induced shift to the right of the [^{3}H]-raclopride versus dopamine competition curves and of the dopamine-induced [^{35}S]-GTPγS binding) in grouped and isolated rats. In particular, the GTPγS binding is being used to study the first step of the intracellular signaling pathway and has been shown to be a suitable method to measure the functional sensitivity of D2 receptors under physiological and pathological circumstances (Sóvágó et al. 2001). Specifically, recent work from our laboratory (Terasmaa et al. 2000) has shown increased D2-stimulated GTPγS binding (supersensitive D2 receptors) in the striatum of rats with lesions of the nigrostriatal dopaminergic pathway. As shown in Fig. 5 and Fig. 6, there were no changes either in the GTPγS-induced shift to the right in the raclopride versus dopamine competition curves or in the dopamine-induced [^{35}S]-GTPγS binding, in the dorsal/ventral striatal membranes of isolated rats relative to grouped rats. These results support our own behavioral findings with quinpirole and suggest that the functional efficacy and potency of D2 receptors in the dorsal/ventral striatum are not modified by rearing conditions. Interestingly, other studies have shown that the inhibition of cAMP through D2 receptor stimulation was reduced in the nucleus accumbens (Hall et al. 1998), but not in dorsal striatum (Jones et al. 1992), of isolated rats. Thus, the possibility that changes of dopamine-induced [^{35}S]-GTPγS binding occur specifically in nucleus accumbens or sub-regions of the nucleus accumbens cannot be ruled out.

In addition to altering locomotor behavior, social isolation has also been reported to induce changes in cognitive function (Robbins et al. 1996; Heidbreder et al. 2000). As shown in the results section, isolated rats had shorter retention latencies 24 h after training in the passive avoidance test. This effect cannot be explained as differences in anxiety levels or spontaneous motor activity prior to conditioning since grouped and isolated rats had the same training latency (see Fig. 2, insert). These results show that social isolation impairs passive avoidance retention (Gardner et al. 1975) and support the fact that rearing rats in a poor social environment can induce cognitive as well as motor behavioral disturbances (Mohammed et al. 1990). The enhanced dopamine release in the nucleus accumbens during footshock shown in isolated rats (Fulford and Marsden 1998) could account, at least in part, for the retention impairment assessed using the passive avoidance paradigm. In fact there is evidence showing that nucleus accumbens is involved in passive avoidance retention (Lorenzini et al. 1995; Roozendaal et al. 2001) and also that dopamine injected into this area of the brain can disrupt passive avoidance performance (Bracs et al. 1984). Future studies will be needed to ascertain whether impaired retention indicates impaired memory and, in that case, which component of the memory process (acquisition, consolidation, retrieval) has become disrupted by the social isolation, as well as to elucidate the specific mechanisms involved.

Previous work has reported that individual and spontaneous locomotor response to novelty can predict alterations in D2 receptors in the dorsal and ventral striatum (Hooks et al. 1994b). In the present study, it is shown that the hyperactivity to novelty induced by social isolation is not correlated with changes in the in vivo function and binding of D2 receptors in dorsal/ventral striatum, which supports the hypothesis that the changes in the motor activity, as well as other behavioral disturbances, induced by rearing rats in isolation are part of a more complex syndrome which probably involves a new neurochemical balance in the interaction of multiple neurotransmitter systems in different areas of the brain (Robbins et al. 1996; Heidbreder et al. 2000). For instance, since lesions in the prefrontal cortex can induce hyperactivity (Lacroix et al. 2000) and enhance nucleus accumbens dopamine function (Brake et al. 2000), changes in prefrontal cortex function may be involved in the behavioral and neurochemical changes observed in isolated rats (Heidbreder et al. 2000; Dalley et al. 2002).

Acknowledgements The research reported in this paper was supported by a Spanish fellowship from the Ministerio de Educación y Ciencia and by the Swedish Research Council.

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