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Long-term social isolation and medial prefrontal cortex: dopaminergic and cholinergic neurotransmission

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Abstract

Rearing rats in social isolation has been suggested as an animal model of schizophrenia, based mainly on the similarity between the attenuation of prepulse inhibition (PPI) in isolated rats and in schizophrenic patients. The medial prefrontal cortex (mPFC) plays a major role in the pathophysiology of schizophrenia. Thus, a postmortem micropunch analysis measuring dopamine (DA), DOPAC (3,4-dihydroxyphenylacetic acid) and homovanillic acid (HVA) in the dorsal and ventral subregion of the mPFC, the caudate putamen (CPu) and nucleus accumbens (NAc) was carried out on socially isolated or group-housed male Sprague–Dawley (SD) rats. Additionally, in vivo microdialysis with D-amphetamine (1 mg/kg ip) stimulation was performed in isolated animals and their controls, examining the ventral mPFC for acetylcholine (ACh), DOPAC and HVA levels. Simultaneously, recording of motor activity was performed. In the neurochemical postmortem tissue analysis we found no difference in any of the brain regions tested between isolated and group-reared animals. Amphetamine increased ACh levels in the mPFC, induced a decrease in DOPAC and HVA levels, and increased motor activity. A close to significant Drug×Housing interaction reflected the fact that the amphetamine-induced decrease of DOPAC was confined to the group-housed animals. In conclusion, social isolation leads only to moderate changes in the dopaminergic system in the mPFC, whereas the cholinergic system remains unaffected.

Keywords: Acetylcholine; Medial prefrontal cortex; Microdialysis; Social isolation; Schizophrenia

1. Introduction

Early lifetime experiences are thought to have a profound impact on the development and maturation of the central nervous system. Depriving rats of social contact with other rats leads to long-lasting alterations in their behavioral profile (Einon and Morgan, 1977; for reviews, see Hall, 1998; Weiss and Feldon, 2001). It has been shown that isolated rats (ISO) exhibit locomotor hyperactivity in the open field (Sahakian et al., 1975; Gentsch et al., 1988; Weiss et al., 2000), impairment in prepulse inhibition (PPI; Geyer et al., 1993; Heidbreder et al., 2000; Weiss et al., 2001b), altered responsiveness to psychostimulants like amphetamine (Sahakian et al., 1975; Jones et al., 1990; Hall et al., 1998), and impaired cognitive functions, such as rule learning (Jones et al., 1991) and attentional shifting (Schrijver and Wurbel, 2001).

In view of the behavioral changes (in particular those suggesting a hyperactive mesolimbic dopamine [DA] system) induced by social isolation, this manipulation has been suggested as an appropriate animal model of schizophrenia (Geyer et al., 1993; Weiss et al., 2001b). The most notable behavioral change induced by social isolation is that of the attenuation of PPI (Geyer et al., 1993; Weiss et al., 2000, 2001b; Weiss and Feldon, 2001). However, the attenuation of PPI following social isolation in rats appears to be strain dependent (Varty and Geyer, 1998; Weiss et al., 2000). Consequently, the present study concentrated on rats of the Sprague–Dawley (SD) strain, which have been shown in our as well as other laboratories to be highly susceptible to this manipulation and PPI attenuation (Bakshi and Geyer, 1999; Geyer et al., 1993; Stevens et al., 1997; Varty and Geyer, 1998; Weiss et al., 2000; Weiss and Feldon, 2001). Indeed, all the animals that were subjects in the present study were part of a previous investigation in which they were shown to have PPI deficits following social isolation (Weiss and Feldon, 2001).

Besides alteration in the nucleus accumbens (NAc), altered function of the medial prefrontal cortex (mPFC) has been implicated in the pathophysiology of schizophrenia (Weinberger, 1995). In schizophrenic patients, cognitive impairments related to prefrontal dysfunction were evident

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and could be correlated using neuroimaging techniques to a reduction in cortical activity, also known as hypofrontality (Weinberger et al., 1988; Andreasen et al., 1992). Especially, the dorsolateral cortex, which is considered to be equivalent to the mPFC in rats, is involved.

Accordingly, neurochemical alterations induced by isolation rearing have been observed in this region (for a review, see Hall, 1998). A reduced cortical DOPAC (3,4-dihydroxyphenylacetic acid)/DA turnover was found in the cortex of ISO rats (Blanc et al., 1980; Heidbreder et al., 2000), reflecting lower DA metabolism.

Furthermore, atypical antipsychotics induced a greater increase of DA in the mPFC in ISO than group-housed GRP rats (Heidbreder et al., 2001). In addition, atypical antipsychotics have been shown to increase cortical acetylcholine (ACh) in the mPFC without an effect in the caudate putamen (CPu) and the NAc (Ichikawa et al., 2002). Therefore, ACh is considered as an important neurotransmitter for the investigation of neurochemical changes in the mPFC after social isolation. Cortical ACh release has also been related to attentional processes (Passetti et al., 2000), which are altered by isolation rearing. We, therefore, were interested in investigating whether long-term (1 year) isolation rearing has an effect on amphetamine-induced ACh release.

In a first experiment, neurochemical postmortem tissue analysis was carried out in the mPFC, the CPu and the NAc. Because the mPFC receives different projections in its ventral and dorsal part (for a review, see Tzschentke, 2001), tissue samples from the dorsal and the ventral mPFC were analyzed separately.

In a second experiment, D-amphetamine was used to study alterations of the dopaminergic and cholinergic neurotransmission in ISO and GRP animals using the in vivo microdialysis technique. Amphetamine releases DA from the vesicles into the cytosol of dopaminergic neurons and reverses the plasma membrane DA transporter (DAT) to enhance synaptic DA levels. It also inhibits DA metabolism via inhibition of monoamine oxidase (MAO), which leads to a decrease of DOPAC and homovanillic acid (HVA) in the extracellular space (Jones et al., 1998), and increases ACh in the mPFC. In addition to the neurochemical measurements, simultaneous determination of activity was carried out.

2. Materials and methods

2.1. Animals

The present study was conducted in male SD rats [Zur:SD(Crl:CD®(SD)BR)], bred in-house at the Research Unit Schwerzenbach, Switzerland. Animals were maintained under standard conditions, in temperature- (21±1.0 °C) and humidity- (55±5%) controlled rooms, on a 12-h reversed light/dark cycle (lights off at 7 a.m.) with free access to standard diet (Nafag 9431, Nafag Ecossan, Gossau, Switzerland) and water ad libitum. All animal studies were carried out in accordance with the European Convention for Animal Care and Use of Laboratory Animals and were approved by the appropriate institutional governmental agency (Kantonales Veterinäramt Zürich, Switzerland).

2.2. Social isolation procedure

For the social isolation procedure, SD rats were mated such that all the mothers gave birth within 1 week. Immediately after birth, all litters were culled in order to have four males and four females in each litter. The mothers and their pups were reared in solid-bottom Macrolon cages (59.0×38.5×20.0 cm) with water and food given ad libitum. At weaning (PND 21), the pups were separated from their mothers and divided and reared either in social isolation (ISO; one rat per cage) or in social groups (GRP; three or four rats per cage) such that each litter contributed only one subject to the GRP and one subject to the ISO condition to avoid a litter effect (Lehmann and Feldon, 2000).

All animals were reared in Macrolon cages with the following dimensions for ISO (48.0×27.0×20.0 cm) and for GRP (59.0×38.5×20.0 cm). Animals were disturbed only for cleaning purposes, which consisted of changing the cage once a week for ISO and twice a week for GRP animals. ISO and GRP rats were housed in the same holding room so that ISO rats maintained visual, auditory and olfactory contact with the other animals throughout the studies. All animals underwent a PPI testing procedure as described and published elsewhere (Weiss and Feldon, 2001) prior to the microdialysis experiment. Male rats were randomly chosen for neurochemical analysis. To achieve stable, social isolation conditions not confounded by the previous tests, the rats were kept for several months undisturbed under isolation or group-housed conditions after completion of behavioral testing and before performing the neurochemical analysis. The microdialysis experiments were carried out in freely moving animals after 1 year of isolation or group housing.

2.3. Postmortem tissue preparation

Rats were decapitated and the brains removed and immediately frozen on dry ice. For dissection (Fig. 1A), the frozen brains were placed ventral side up in an ice-chilled rat brain matrix (ASI, Warren MI, USA) with slits spaced at 1 mm. The brain was first fixed in the matrix by one ice-chilled razor blade placed in the slit about 2 mm anterior to the end of the olfactory tract, which can easily be identified on the brain's ventral surface and whose location corresponds to a coordinate of about 3 mm anterior to bregma in the rat brain atlas of Paxinos and Watson (1998), and by another blade placed in the slit next to the beginning of the cerebellum. Three additional ice-chilled razor blades, spaced at 2-mm intervals from the first, most anterior razor blade were then placed to obtain three 2-mm coronal brain sections from which the forebrain regions of interest could be punched and whose posterior sides corre-

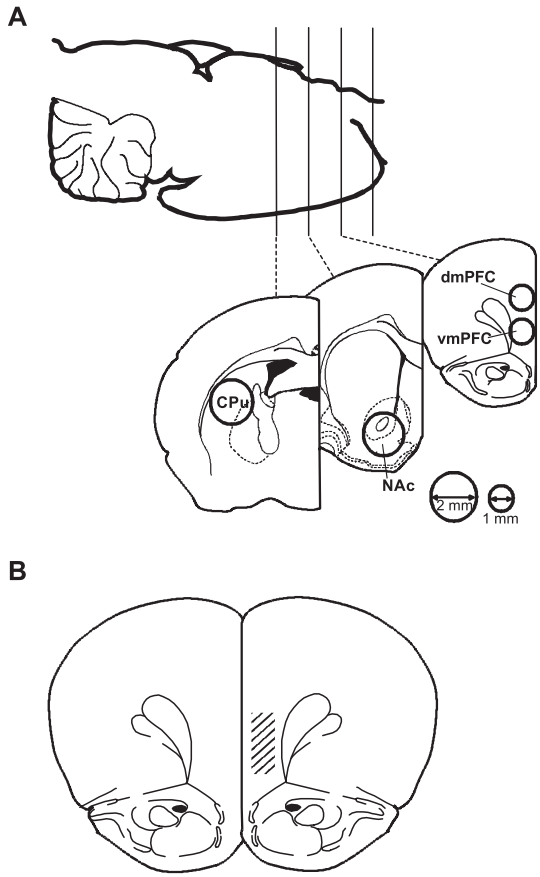

Fig. 1. (A) Regions of the postmortem tissue preparation and (B) the in vivo microdialysis probe placement area (designated area) in the ventral mPFC. (A) The locations of the razor blades used to cut three adjacent coronal slices are indicated in a sagittal view of the rat brain with the ventral surface up (top); forebrain regions of interest were dissected by pushing micropunch needles of 1- or 2-mm diameter into the posterior face of the coronal slices as indicated (bottom); dmPFC, dorsal mPFC; vmPFC, ventral mPFC; CPu, caudate putamen; NAc, nucleus accumbens.

sponded to approximately 3, 1 and −1 mm from bregma according to the atlas of Paxinos and Watson. The three 2-mm coronal sections were placed posterior side up onto an ice-chilled plate covered with filter paper. To obtain the desired tissue samples, punch needles of diameter 2 or 1 mm and connected to a pressure system (ASI) were pushed into the appropriate locations on the posterior side of the sections (Fig. 1A), withdrawn, and the tissue samples were ejected by a slight pressure increase into preweighed and ice-chilled 1.5-ml Eppendorf tubes. The dorsal mPFC and ventral mPFC were punched with needles of 1-mm diameter and the CPu and the NAc were punched with needles of 2-mm diameter. After weighing, 200 μl of ice-cold 0.4 M $HClO_4$ was added to each tissue sample, the samples were homogenized by slight sonification for about 10 s, centrifuged at 15,000×*g* and 4 °C for 20 min, and then filtered into CMA autosampler vials for consecutive HPLC analysis. Injection volume was 50 μl for samples from the dorsal mPFC and the ventral mPFC and 10 μl for samples from the CPu and the NAc. HPLC analysis was performed as described below.

2.4. Surgical procedure for in vivo microdialysis

Animals were anaesthetized with sodium pentobarbital (60 mg/kg ip) and atropine (0.05 mg/kg sc) and were then mounted on a stereotaxic apparatus (David Kopf, Tujunga, CA, USA) with the upper incisor bar set below the interaural line to obtain a flat-skull position. The skull was exposed and a hole drilled for unilateral placement of an intracerebral guide cannula (Microbiotech MAB 4.9.IC, Stockholm, Sweden). The coordinates aiming at the ventral part of the mPFC were as follows: +3.2 mm anterior to bregma, +0.5 mm lateral to midline and −3.5 mm ventral to the skull, according to the brain atlas of Paxinos and Watson (1998). Following surgery, animals were housed individually in Macrolon cages (50×25×20 cm) and allowed at least 1 day to recover before the microdialysis procedure.

2.5. In vivo microdialysis procedure

Concentric microdialysis probes (MAB4.9.2.CU, 2 mm cuprophane membrane length, 0.24 mm diameter, Microbiotech) were introduced into the guide cannula in awake rats on the day of the experiment. The microdialysis probes were perfused at a flow rate of 2.5 μl/min with artificial cerebrospinal fluid containing 147.0 mM NaCl, 4.0 mM KCl, 1.0 mM $MgCl_2$, 1.3 mM $CaCl_2$ and 0.5 μM neostigmine bromide to prevent ACh degradation. Dialysis samples were automatically collected with a microfraction collector (CMA/142, CMA/Microdialysis, Stockholm, Sweden) every 20 min in a vial containing 10 μl hydrochloric acid (0.1 M). After equilibration time (180 min), four dialysate samples were collected that were regarded as 100% baseline. Rats were then injected with either amphetamine (1 mg/kg ip calculated as free base) or saline and additional six dialysate fractions were sampled. The data were not corrected for the in vitro recovery, which was 8–10% for DA metabolites and 10–12% for ACh measured at room temperature.

After the microdialysis experiment, animals were deeply anesthetized with sodium pentobarbital and transcardially perfused with 200 ml 0.9% NaCl solution followed by 500 ml 4% formalin diluted in 0.1 M phosphate-buffered saline (PBS). The brains were removed and placed in a cryoprotectant solution of 30% sucrose in PBS. Brains were sectioned on a freezing microtome at a thickness of 50 μm and stained with cresyl violet. Only animals with correct probe placement (see Fig. 1) were included in the analysis.

2.6. Motor activity measurement during microdialysis procedure

A miniature video camera with a wide-angle (100°) 2.5-mm lens (VPC-465B, CES, Zurich, Switzerland) was placed above each microdialysis cage. All experimental sessions were video recorded under dimmed room light.

Individual video inputs from four microdialysis cages (diameter 36 cm, height 40 cm) were analyzed by computer software (written by P. Schmid) for automated motor activity quantification as described previously (Richmond et al., 1998). In brief, a Visual Basic program combined with Activex functions implemented from an image analysis program (WIT, version 5.3) compared adjacent frames from the online signal of the camera. A four-quarter frame was recorded every second, and changes between two adjacent frames were calculated as follows: Each pixel was compared with the same pixel from the previous frame. If the difference in brightness for a given pixel was more than 8% (this threshold was chosen to achieve optimal noise suppression) it was defined as a "changed pixel." The percentage of changed pixels was used as a measure of motor activity. Motor activity was monitored in 1-s intervals and averaged over 20-min time blocks to refer to the corresponding microdialysate fractions.

2.7. Chromatographic analysis of postmortem tissue and microdialysis samples

Filtered postmortem samples were analyzed for DA, DOPAC and HVA using high-performance liquid chromatography (HPLC) combined with electrochemical detection, described below for catecholamine detection. The microdialysis samples were analyzed for DOPAC and HVA using HPLC combined with electrochemical detection and for ACh using post column derivatization and subsequent electrochemical detection. Our intention was also to measure extracellular DA. However, we did not detect high enough DA levels for reliable analysis in the mPFC because of the splitting of the microdialysis sample for simultaneous measurement of catecholamines and ACh, and probably because of the age of the rats. We therefore excluded the DA data that were often below the detection limit, and focused on DA metabolite levels. A refrigerated autoinjector (CMA/200, CMA/Microdialysis) equipped with a 10-port high-pressure valve with two sampling loops was used for injection of 19.7-μl aliquots into two separate HPLC systems from the same sample vial to reduce loss of dialysate volume. For postmortem tissue, a 50-μl loop was inserted to increase the injection volume. In the catecholamine system chromatographic separation was performed using a reversed-phase column (Chrompack; 100×3.0 (ID) mm) with precolumn (10×2.0 (ID) mm) packed with derivatized silica material (ChromSpher B, 5-μm particle size). The mobile phase consisted of 34.88 mM citric acid, 54.37 mM sodium acetate, 0.67 mM disodium ethylenediamine tetraacetate, and 0.46 mM 1-octanesulfonic acid sodium salt to which 2% methanol (vol/vol) was added (final pH 4.1), filtered through a 0.22-μm filter, degassed under vacuum, and delivered at a flow rate of 1.0 ml/min using an HPLC solvent-delivery system (Rheos 4000, Flux Instruments, Basel, Switzerland). The oven of the electrochemical amperometric detector (Decade, ANTEC Leyden, Leiden, the Netherlands) maintained a constant temperature of 30 °C for the column and a glassy carbon working electrode, which was set at +750 mV versus Ag/AgCl reference electrode.

Acetylcholine determination was carried out with a Chrompack acteylcholine kit (Varian, CA, USA). A Chrompack choline analytical column (100×3.0 (ID) mm) packed with chemically derivatized silica material (5-μm particle size) was used for separation. For postcolumn derivatization an immobilized enzyme reactor (Chromsep IMER) loaded with ACh esterase and choline oxidase allowed the stoichiometric conversion of ACh to acetic acid and choline, and choline to betaine and hydrogen peroxide. The hydrogen peroxide was then electrochemically determined using an electrochemical amperometric detector (Decade, ANTEC-Leyden) via oxidation on a platinum working electrode set at +500 mV versus an Ag/AgCl reference electrode at a temperature of 30 °C. The mobile phase consisted of 0.2 M potassium phosphate dibasic, 1 mM tetramethylammonium hydroxide (pH of 8.0 adjusted with 0.2 M potassium phosphate monobasic). After filtration (0.22-μm pore size) and degassing under vacuum the mobile phase was delivered at a flow rate of 0.6 ml/min onto the column using an HPLC solvent-delivery system (Rheos 4000, Flux Instruments).

A chromatography workstation (Chromeleon, Dionex, Olten, Switzerland) was used for data acquisition and calculation. The position and areas of the peaks of the compounds of interest were compared with external calibrating standard solutions. The detection limit of the catecholamine system was 1 nM for DA, DOPAC and HVA and 20 nM for ACh using an injection volume of 20 μl.

2.8. Drugs and chemicals

Citric acid, sodium acetate, ethylenediaminetetraacetic acid disodium salt, 1-octanesulfonic acid sodium salt, sodium chloride, potassium chloride, magnesium chloride, calcium chloride, methanol, diethylamine, tetramethylammonium hydroxide, potassium phosphate, D-amphetamine sulfate and phosphoric acid were of analytical grade and were obtained from Fluka Chemie, Buchs, Switzerland. D-Amphetamine was dissolved in 0.9% saline solution for intraperitoneal injection (1 ml/kg injection volume).

2.9. Data analysis

For the postmortem analysis in the mPFC, levels of DA, DOPAC and HVA were analyzed by ANOVA using housing (grouped/isolated) as a between-subjects factor and location (dorsal mPFC/ ventral mPFC) as a within-subjects factor. Samples from the CPu and NAc were analyzed using only housing as a between-subjects factor.

For the microdialysis samples, basal levels of DOPAC, HVA and ACh were calculated as the average of the levels of the first four dialysate fractions and were analyzed by ANOVA using housing (grouped/isolated) and drug (saline/

amphetamine) as between-subjects factors. The drug effects on baseline levels were presented to show that there are no differences between the groups prior to drug administration. Amphetamine-induced changes in neurotransmitters and metabolites were calculated as percentage of basal level and analyzed by ANOVA using housing (grouped/isolated) and drug (saline/amphetamine) as between-subjects factors and dialysate fractions as a within-subjects repeated measurement factor. Only dialysates after amphetamine injection were used for the analysis of the amphetamine effect. Motor activity was measured during the microdialysis experiment and the motor activity counts were presented in 20-min time blocks corresponding to the collection intervals of microdialysates. They were analyzed by ANOVA using housing (grouped/isolated) and drug (saline/amphetamine) as between-subjects factors and time intervals as a within-subjects repeated measurement factor. For post hoc comparisons, Fisher's least significant difference (PLSD) test was used. Differences were considered significant only when P values were less than .05.

3. Results

3.1. Postmortem analysis

3.1.1. Effects of social isolation on tissue concentration of DA, DOPAC and HVA

In the mPFC, a significant difference in DA content between the dorsal and ventral mPFC [$F(1,13)=16.18$, $P<.01$], with more DA in the ventral than in the dorsal mPFC (Table 1), was obtained. There was also a close to significant interaction of Housing×Location [$F(1,13)=3.46$, $P<.09$], reflecting higher DA values that seemed to be higher in the ventral mPFC of ISO rats compared to GRP rats; because of the high variance this effect did not reach the acceptable level of significance. No differences were found between ISO and GRP rats in DOPAC, HVA levels and DA turnover, calculated as (DOPAC+HVA)/DA. In the CPu and the NAc, there were no differences in DA, DOPAC, HVA and DA turnover between GRP and ISO rats.

Table 1
Effect of social isolation on postmortem neurochemistry

	Grouped	Isolated
DA (mean±S.E.M.)		
dmPFC	0.13±0.03	0.12±0.02
vmPFC	0.17±0.03	0.25±0.03
CPu	10.81±0.84	9.37±1.39
NAc	5.89±0.38	6.14±0.55
DOPAC (mean±S.E.M.)		
dmPFC	0.18±0.03	0.17±0.03
vmPFC	0.21±0.03	0.28±0.04
CPu	2.40±0.26	2.12±0.37
NAc	2.57±0.26	2.70±0.17
HVA (mean±S.E.M.)		
dmPFC	0.10±0.02	0.10±0.02
vmPFC	0.12±0.03	0.15±0.02
CPu	0.72±0.07	0.73±0.14
NAc	0.59±0.06	0.70±0.05
(DOPAC+HVA)/DA		
dmPFC	3.11±0.73	2.54±0.54
vmPFC	2.16±0.30	2.53±0.90
CPu	0.29±0.02	0.30±0.02
NAc	0.54±0.04	0.59±0.06

Tissue concentration of DA, DOPAC, HVA and DA turnover from the dorsal and ventral mPFC, the CPu and the NAc measured in nanograms per milligram wet tissue weight. Data are mean±S.E.M. of $n=7-8$ rats.

3.2. Motor activity

3.2.1. Effects of social isolation on spontaneous (basal) activity

Before amphetamine injection motor activity levels did not differ between main treatment groups (housing [$F(1,32)=0.065$, $P=.8$], drug [$F(1,32)=0.76$, $P=.4$]; Fig. 2.)

3.2.2. Effects of social isolation on amphetamine-induced hyperactivity

Amphetamine treatment increased motor activity from 0.7 arbitrary units (a.u.) up to about 7.2 a.u. in both housing treatment groups (Fig. 2). ANOVA yielded a highly significant effect of drug [$F(1,32)=65.65$, $P<.001$], time intervals [$F(5,160)=7.71$, $P<.001$], and also a highly significant Drug×Time Intervals interaction [$F(5,160)=9.01$, $P<.001$]. However, no Housing×Drug×Time Intervals interaction was seen [$F(5,160)=1.1$, $P=.4$], suggesting that social isolation did not modify animal's sensitivity to a low dose of amphetamine.

3.3. In vivo microdialysis

3.3.1. Effects of social isolation on extracellular DOPAC levels

Basal levels of DOPAC were not significantly different between housing conditions [$F(1,27)=1.23$, $P=.3$]. However, the drug effect approached significance [$F(1,27)=3.94$, $P<.06$], suggesting higher DOPAC levels in the AMPH treatment groups, irrespective of housing conditions. Basal levels were 8.4±2.4 nM for GRP SAL, 15.6±4.8 nM for GRP AMPH, 10.8±1.2 nM for ISO SAL and 25.2±7.2 nM for ISO AMPH.

Following the administration of AMPH or vehicle, there was a gradual reduction of DOPAC levels over the six dialysate measurements (main effect of dialysate fractions [$F(1,135)=2.78$, $P<.025$] irrespective of housing conditions) (Fig. 3A). This reduction tended to be more in AMPH-treated animals. In addition, the close to significant interaction of Drug×Housing [$F(1,27)=3.82$, $P=.06$] reflected that there was a reduction in DOPAC levels seen following the administration of AMPH in the GRP rats (average 21.8±3.9% reduction, $P<.05$), whereas the ISO rats showed no such reduction in DOPAC levels.

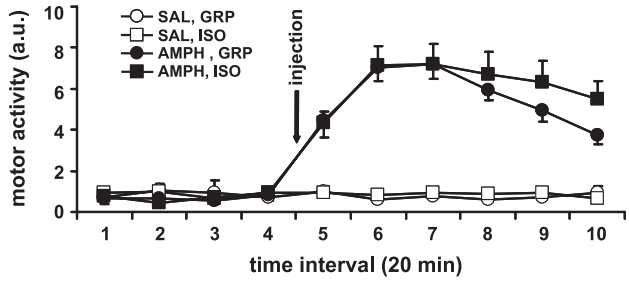

Fig. 2. Effects of amphetamine on motor activity in grouped (GRP) and isolated (ISO) rats. Motor activity was quantified using an automated video observation system and displayed in arbitrary units (a.u.) in 20-min time intervals. Time intervals 1 to 4 represent basal levels before a systemic amphetamine (AMPH; 1 mg/kg) injection. The control group (SAL) received a saline injection. Data are mean ± S.E.M. of n=7–11 rats.

3.3.2. Effects of social isolation on extracellular HVA levels

In HVA basal levels, the effect of housing was close to significance [$F(1,31)=3.72$, $P<.065$] suggesting higher HVA basal levels in isolated animals. However, there was no effect of drug [$F(1,31)=0.18$, $P=.2$]. The basal levels were 25.2±4.8 nM for GRP SAL, 31.2±4.8 nM for GRP AMPH, 34.8±3.6 nM for ISO SAL and 46.8±8.4 nM for ISO AMPH.

HVA levels remained stable following saline injection but were gradually decreased following AMPH injection

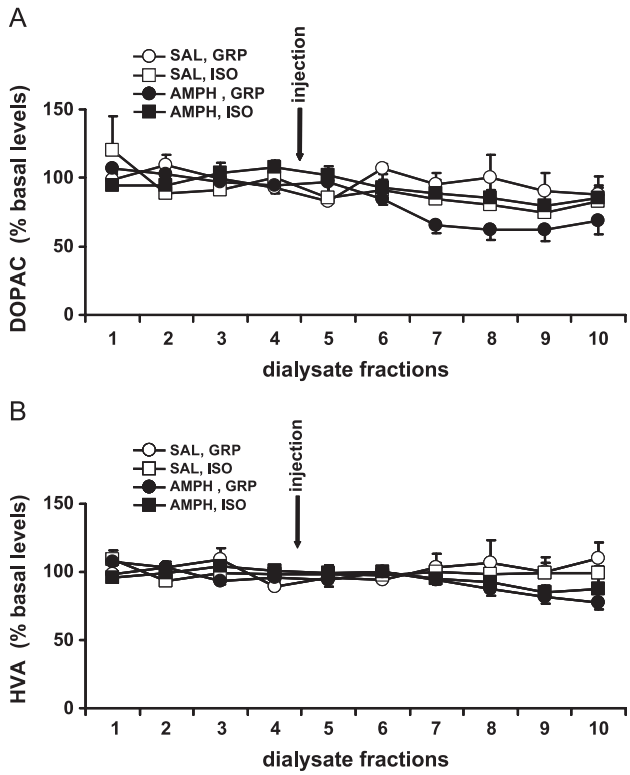

Fig. 3. Effects of amphetamine on (A) DOPAC and (B) HVA in grouped (GRP) and isolated (ISO) rats. Dialysate fractions from the mPFC were collected every 20 min. Fractions 1 to 4 represent basal levels. After Fraction 4, amphetamine (1 mg/kg; AMPH) or saline (SAL) was administered. Data are mean ± S.E.M. of n=6–11 rats.

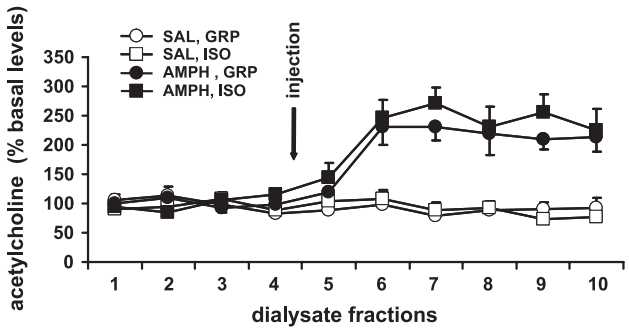

Fig. 4. Effects of amphetamine on ACh transmission in grouped (GRP) and isolated (ISO) rats. Dialysate fractions from the mPFC were collected every 20 min. Fractions 1 to 4 represent basal levels. After Fraction 4, amphetamine (AMPH; 1 mg/kg) or saline (SAL) was administered. Data are mean±S.E.M. of n=6–11 rats.

(Fig. 3B). This was supported by the Drug×Dialysate Fraction interaction [$F(5,155)=5.61$ $P<.001$). No other main effects or interactions were seen.

3.3.3. Effects of social isolation on extracellular ACh levels

Basal levels of ACh were not significantly different between housing conditions [$F(1,31)=0.70$, $P=.4$] and drug [$F(1,31)=0.69$, $P=.4$]. They were 34.8±8.4 nM for GRP SAL, 38.4±3.6 nM for GRP AMPH, 38.4±8.4 nM for ISO SAL and 45.6±6 nM for ISO AMPH (Fig. 4).

After amphetamine injection, ANOVA yielded an interaction of Drug×Dialysate Fractions [$F(5,155)=6.68$, $P<.001$] combined with main effects of drug [$F(1,31)=34.04$, $P<.001$] and dialysate fractions [$F(5,155)=5.06$, $P<.001$], reflecting an increase in ACh level after AMPH injection only in the AMPH-treated animals (main effect of dialysate fractions in AMPH rats [$F(5,100)=10.49$, $P<.001$]) irrespective of housing condition. No interactions of Housing×Dialysate Fractions [$F(5,155)=0.23$, $P=.95$] and Housing×Drug×Dialysate Fractions [$F(5,155)=0.4$, $P=.9$] were found.

4. Discussion

In the present study, the neurochemical consequences following long-term social isolation were investigated. The postmortem study showed a higher level of DA content in the ventral mPFC than in the dorsal, with no differences in DA metabolite levels. Social isolation attenuated the AMPH-induced reduction of DOPAC. However, HVA and ACh levels were unaffected by social isolation.

4.1. Effect of isolation rearing on tissue concentration of DA and DA metabolites

The concentrations of DA and DA metabolites were in the range of that found in the literature (Jones et al., 1992; Matthews et al., 2001). There were regional differences in

the content of the substances measured, with the mPFC having the lowest concentration of DA and its metabolites and the CPu the highest. The mPFC had the highest DA turnover. Comparing subregions, the ventral mPFC had higher amounts of DA than the dorsal mPFC. Social isolation did not affect the brain tissue concentration of DA and its metabolites, although a tendency towards higher DA levels in ISO rats compared to GRP rats in the ventral mPFC was detected. The isolation effect might be masked due to the high variance and would be in line with former observations. Isolation rearing was previously found to enhance DA in the mPFC (Jones et al., 1992). However, this effect was diminished by exposure to the experimental conditions (Jones et al., 1991). Therefore, in our study the previous measurement of PPI and the high age of the rats might increase the variability and diminish the differences between our ISO and GRP animals. Other studies found decreased DA turnover (Blanc et al., 1980; Heidbreder et al., 2000) in the mPFC.

4.2. Effect of isolation rearing on basal and amphetamine-induced motor activity

No isolation-induced spontaneous hyperlocomotor activity was observed in the present study. This is not surprising and is consistent with previous studies, where isolated SD rats showed no increase in spontaneous motor activity in contrast to studies undertaken with other strains (Weiss et al., 2000). This emphasizes the idea that increased spontaneous hyperlocomotor activity is not necessarily associated with social isolation. However, these animals showed a reduction in PPI (Weiss and Feldon, 2001) that has more relevance for prefrontal dysfunction than hyperlocomotor activity (Lacroix et al., 2000).

Amphetamine-induced hyperactivity was not significantly different between ISO and GRP SD rats. This also agrees with a previous finding reported by our group (Weiss et al., 2001a) and others (Sahakian et al., 1975; Bardo et al., 1995). However, in other strains, such as Wistar and Lister hooded, isolation rearing enhanced the response to amphetamine (Jones et al., 1990; Ahmed et al., 1995; Smith et al., 1997). In our experiment, ISO animals seemed to have a more prolonged response to AMPH. However, this effect was not significant, but tended to the direction observed in other rat strains.

4.3. Effect of social isolation on basal and amphetamine-induced changes in DOPAC and HVA levels

In the analysis of DOPAC basal level the main effect of drug was close to significance. This probably reflected a sampling error due to an inadvertent result of group assignment, inasmuch as both treatment groups were treated in the same way during baseline sampling. For the basal levels of HVA, the effect of housing condition was also close to significance. Another microdialysis study, investigating the NAc and the CPu, found no changes in basal levels of DOPAC and HVA between ISO and GRP rats (Jones et al., 1992). After amphetamine treatment, extracellular DOPAC levels were only decreased in GRP animals. The decrease of metabolites after AMPH administration is considered to at least partly result from MAO inhibition by AMPH (Miller et al., 1980). The amphetamine-induced decrease of DOPAC seemed to be of lesser magnitude in ISO rats. Therefore, it is likely that ISO rats show an altered dopaminergic metabolism due to social isolation. In a study by Jones et al. (1992), amphetamine induced a greater decrease in DOPAC in the CPu in ISO animals than in GRP animals. This was accompanied by an increased response of DA to AMPH. The reciprocal relationship between the mPFC and the subcortical areas is reflected by an increase of striatal DA activity and a reduction in DA activity in the mPFC (Pycock et al., 1980). Theoretically, reduced blood flow in the mPFC, which leads to reduced delivery and distribution of AMPH, may reduce the amphetamine effect in ISO rats. This would also fit with the human data showing reduced cerebral blood flow in the cortical areas in schizophrenic patients (Weinberger et al., 1988; Andreasen et al., 1992). Furthermore, social isolation might affect the autoreceptor function in the mPFC leading to reduced release and synthesis of DA. In the hippocampus of social isolated rats alterations in α_2 autoreceptor function led to a reduced tail-pinch-induced norepinephrine release (Fulford and Marsden, 1997). Since in the mPFC the norepinephrine transporter (NET) and DAT have been shown to be an important uptake system for DA in the cortex (Carboni et al., 1990) an additional explanation would be adaptive changes caused by social isolation in DAT and NET expression.

4.4. Effect of social isolation on basal and amphetamine induced changes in ACh level

Basal levels of ACh were not different between housing conditions. This is in line with activity of the ACh-synthesizing enzyme choline acetyltransferase in the mPFC not differing between ISO and GRP rats (Jones et al., 1991). Additionally, Morley and Worsham (1978) found no difference in the locomotor response to scopolamine and physostigmine between ISO and GRP animals. Amphetamine administration led to a significant increase of cortical ACh (Dalley et al., 2002), but there was no evidence of differences in the levels of ACh between GRP and ISO rats.

The neurochemical findings of the postmortem study and also the basal extracellular levels of the microdialysis experiment indicate that there is no difference between ISO and GRP rats under drug-free conditions. However, after amphetamine challenge, moderate changes in the response of DOPAC level became evident. Thus, the effects of social isolation appear to become apparent after stimulation of the dopaminergic system.

In conclusion, our findings suggest that social isolation leads only to moderate alteration in the dopaminergic

system in the mPFC, whereas the cholinergic system remains unaffected. This emphasizes and elucidates the behavioral effects on PPI (Weiss and Feldon, 2001) obtained before in the same batch of animals.

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