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Dopaminergic and Serotonergic Function Following Isolation Rearing in Rats: Study of Behavioural Responses and Postmortem and In Vivo Neurochemistry

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JONES, G. H., T. D. HERNANDEZ, D. A. KENDALL, C. A. MARSDEN AND T. W. ROBBINS. *Dopaminergic and serotonergic function following isolation rearing in rats: Study of behavioural responses and postmortem and in vivo neurochemistry.* PHARMACOL BIOCHEM BEHAV 43(1) 17-35, 1992.—This series of experiments compared isolation-reared and socially reared rats for their locomotor activity, behavioural stereotypy, and monoamine function both postmortem and in vivo using intracerebral dialysis. In Experiment 1, isolates showed an altered time course of locomotor activity following *d*-amphetamine sulphate (AMPH) administration (0.5, 2.0, 3.0, or 5.0 mg/kg, SC). Isolation-reared rats also showed increased sensitivity to the sedative effects of a low dose of apomorphine hydrochloride (0.1 mg/kg) but did not differ from social controls following higher doses of the drug (0.5, 1.5, or 3.0 mg/kg, SC). Isolates showed a decrease in the intensity of apomorphine-induced stereotyped behaviours but no change in stereotypy induced by AMPH. In Experiment 2, isolates had higher postmortem dopamine (DA) concentrations and an altered asymmetry in DA function in the medial prefrontal cortex (PFC) but not in the nucleus accumbens (NAC) or caudate putamen (CPu). Isolated rats also had a lower 5-hydroxyindoleacetic acid (5-HIAA)/5-hydroxytryptamine (5-HT) ratio in the NAC (but not in the PFC or CPu) compared to controls. Experiment 3 used intracerebral dialysis to examine monoamine function in vivo following isolation rearing. Isolates showed greater increases in extracellular DA and greater decreases in DOPAC in response to 2 mg/kg AMPH SC in both the NAC and CPu. There were no apparent differences in the perfusate concentrations of either dopamine (DA), dihydroxyphenylacetic acid (DOPAC), or homovanillic acid (HVA) prior to drug administration. However, consistent with the results of Experiment 2, isolates had a reduced basal perfusate concentration of 5-HIAA from the NAC but not from the CPu. Experiment 4 measured postsynaptic DA function in CPu tissue slices following isolation. Isolation rearing did not affect cAMP accumulation in response to stimulation of D_1 DA receptors by DA (0, 2.7, 9, or 30 μM). In addition, isolation rearing did not affect the coupling between D_1 and D_2 receptors, as measured by the increase in cAMP accumulation with 1 μM 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1 *H*-3-benzazepin (SK&F 38393) and its reduction by 10 μM quinperole hydrochloride (LY 171555). These results are discussed in terms of the possible relationship between these neurochemical findings and the behavioural disturbances following isolation rearing of rats.

Isolation rearing Locomotor activity Stereotypy Nucleus accumbens Caudate putamen
Prefrontal cortex Dialysis Dopamine Serotonin

MANY of the behavioural disturbances following isolation rearing in rats and other species have specifically implicated a role for a central monoamine substrate. Isolates are generally hyperactive (28,29,40,44,66) and show a shift to the left in the dose-response curve to *d*-amphetamine (AMPH) (40). Considering the robust nature of some of the behavioural effects of isolation, for example, the locomotor hyperactivity (28, 29,40,44,66) and impairments in response inhibition (41,66,

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67,68,72), there have been relatively few comprehensive studies of the possible neural basis for these effects. The current series of experiments is designed to investigate more fully the function of brain monoamines following isolation rearing in the rat. In Experiment 1, the effects of isolation rearing on locomotor activity and behavioural stereotypy following either *d*-amphetamine or apomorphine were examined to confirm that the rearing conditions used in the current series of experiments produced comparable results to previous findings (27,40,82). Experiments 2, 3, and 4 examined monoamine function following isolation rearing. Previous studies of the function of monoamines in isolates have been largely limited to a single neurotransmitter (69,93,104), and few studies have measured more than one neurotransmitter simultaneously in several brain regions in experimentally naive rats (98). In Experiment 2, postmortem monoamine and metabolite concentrations in the medial prefrontal cortex (PFC), nucleus accumbens (NAC), and anterior (aCPu) and posterior (pCPu) caudate putamen were determined in both socially reared and isolation-reared rats. Experiment 3 measured monoamine function in the NAC and CPu in vivo using intracerebral dialysis procedures (101). In Experiment 4, the effects of isolation rearing on postsynaptic dopamine (DA) receptor function in the CPu were assessed.

EXPERIMENT 1

Isolated and socially reared rats were compared on their locomotor and stereotyped response to the direct DA agonist, apomorphine (1), and the indirect DA agonist, AMPH (64,65). These two drugs were chosen as they provide information on pre- and postsynaptic DA function and to add to the literature on the response to psychomotor stimulants following isolation (22,33,63,82). The behavioural response to these drugs provide information on possible neural changes following isolation. For example, the locomotor-stimulating properties of AMPH primarily depend upon DA function in the NAC, and behavioural stereotypy induced by higher doses of the drug is dependent upon CPu DA (50). In addition, different doses of apomorphine are thought to produce behavioural effects based upon the stimulation of different populations of DA receptors. Low doses of apomorphine produce locomotor hypoactivity and sedation, possibly due to stimulation of presynaptic autoreceptors (9,20), whereas higher doses result in hypermobility and stereotyped behaviours (2,62) due to stimulation of both D_1 and D_2 DA receptors located postsynaptically (102).

Alteration of the locomotor response to DA agonists in isolates might be expected given their hyperactivity in arousing situations (28,29,40,44,66). Furthermore, social isolation has also been considered to be stressful (17,34) and repeated exposure to conventional stressors, such as foot-shock, can potentiate the locomotor response to AMPH (35). Similarly, behavioural stereotypy induced by psychomotor stimulants can also be influenced by exposure to stress (3,8) and by the level of control that an organism has over its environment (60).

METHOD

Subjects

The subjects were 16 female Lister hooded rats (Harlan Olac, U.K.). Eight were reared in isolation and 8 in social groups. Upon arrival at the laboratory, aged 21 days, they were divided into the two rearing condition groups counterbalanced by body weight. Isolation-reared rats were housed individually in cages 45 × 20 × 20 cm high. Socially reared rats were initially housed 8–12 rats per cage (56 × 38 × 18 cm high), which was reduced to 6 rats per cage after 3 weeks. All cages were of plastic construction with steel grid floors and underhanging sawdust trays (North Kent Plastic Cages Ltd., Dartford, U.K.).

All rats were housed in a colony room maintained at 21° C on a 12 L : 12 D (light on 0700 h) with free access to food and water and could see, hear, and smell other rats.

Drugs

d-Amphetamine sulphate (Sigma Chemical Co., St. Louis, MO) was prepared in 0.9% saline and apomorphine hydrochloride (Sigma) in saline containing 0.01% ascorbic acid as an antioxidant. All solutions were prepared immediately before injection and administered in a volume of 0.5 ml/kg body weight.

Procedure

Locomotor activity was monitored in a bank of individual wire mesh photocell cages (40 × 25 × 18 cm). Each cage was fitted with two parallel horizontal infrared beams, 1 cm above the floor, spaced equally along the long axis of the cage. Interruption of either beam resulted in an incremental count for that cage, registered by on-line input to a microcomputer, housed in an adjacent room. All tests for locomotor activity were conducted between 0800–1600 h.

Subjects entered the experiment 12 weeks after arriving in the laboratory. Rats were placed in photocell cages and after 1-h habituation period injected with either 0.5, 2.0, 3.5, or 5.0 mg/kg AMPH or 0.1, 0.5, 1.5, or 3.0 mg/kg apomorphine or vehicle. All subjects received all doses of both drugs. Injections were made SC in the back of the neck. Administration of each dose of drug was in a pseudorandom order with at least 3 days between each injection. Doses of AMPH were administered before doses of apomorphine. Locomotor activity was monitored for 4 h after injection of AMPH and for 1 h after apomorphine injection.

At 30, 60, 90, and 120 min after injection of AMPH (3.5 or 5.0 mg/kg), behavioural stereotypy was rated by an observer blind to the experimental groups. Stereotypy was also rated 60 min after 2 mg/kg AMPH and at 30 and 60 min after apomorphine (1.5 or 3.0 mg/kg). Stereotypy was rated on a 0- to 6-point scale described in detail elsewhere (42).

Data Analysis

The results for AMPH locomotor activity were subjected to a square-root transformation to achieve homogeneity of variance (107). The untransformed data were also analyzed and showed highly comparable effects. Since the stereotypy rating scale did not constitute an interval or ratio scale of measurement, the "information statistic" (54) was used. This nonparametric test assesses the inhomogeneity of distributions in a matrix of data and is distributed as χ^2.

RESULTS

Isolated rats gained weight faster than controls, $F(4, 64) = 12.61$, $p < 0.001$, and were hyperactive on the first exposure to the photocell test cages, $t(16) = 3.12$, $p < 0.01$.

Isolation-reared rats did not significantly differ from social controls in their locomotor activity scores for the 1-h habituation period prior to the first drug injection, although there

was a trend for them to be more active, $F(1, 14) = 4.20$, n.s. During all other habituation periods, there were no significant differences between the two rearing condition groups.

Figure 1 shows the effect of rearing rats in isolation on the locomotor response to AMPH administration. There was no main effect of rearing condition, $F(1, 14) = 1.38$, n.s., or rearing × dose interaction, $F(4, 56) = 0.93$, n.s. However, isolation rearing did alter the time course of the locomotor-stimulating effects of AMPH, as indicated by a significant three-way interaction between rearing, dose, and time, $F(28, 392) = 3.03$, $p < 0.001$. As is evident from Fig. 1, there is an apparent shift to the left in the time course of locomotor activity in isolated rats.

Isolation rearing only affected the locomotor response to apomorphine at 0.1 mg/kg, rearing × dose, $F(4, 56) = 2.83$, $p = 0.033$. As indicated in Fig. 2, this dose of apomorphine dramatically reduced locomotor activity in both groups during the second 10-min time bin. This effect was significantly prolonged in isolates, as indicated by a significant three-way interaction between rearing, dose, and time, $F(20, 280) = 2.01$, $p = 0.007$. There were no differences between the rearing condition groups either following saline or at any other dose

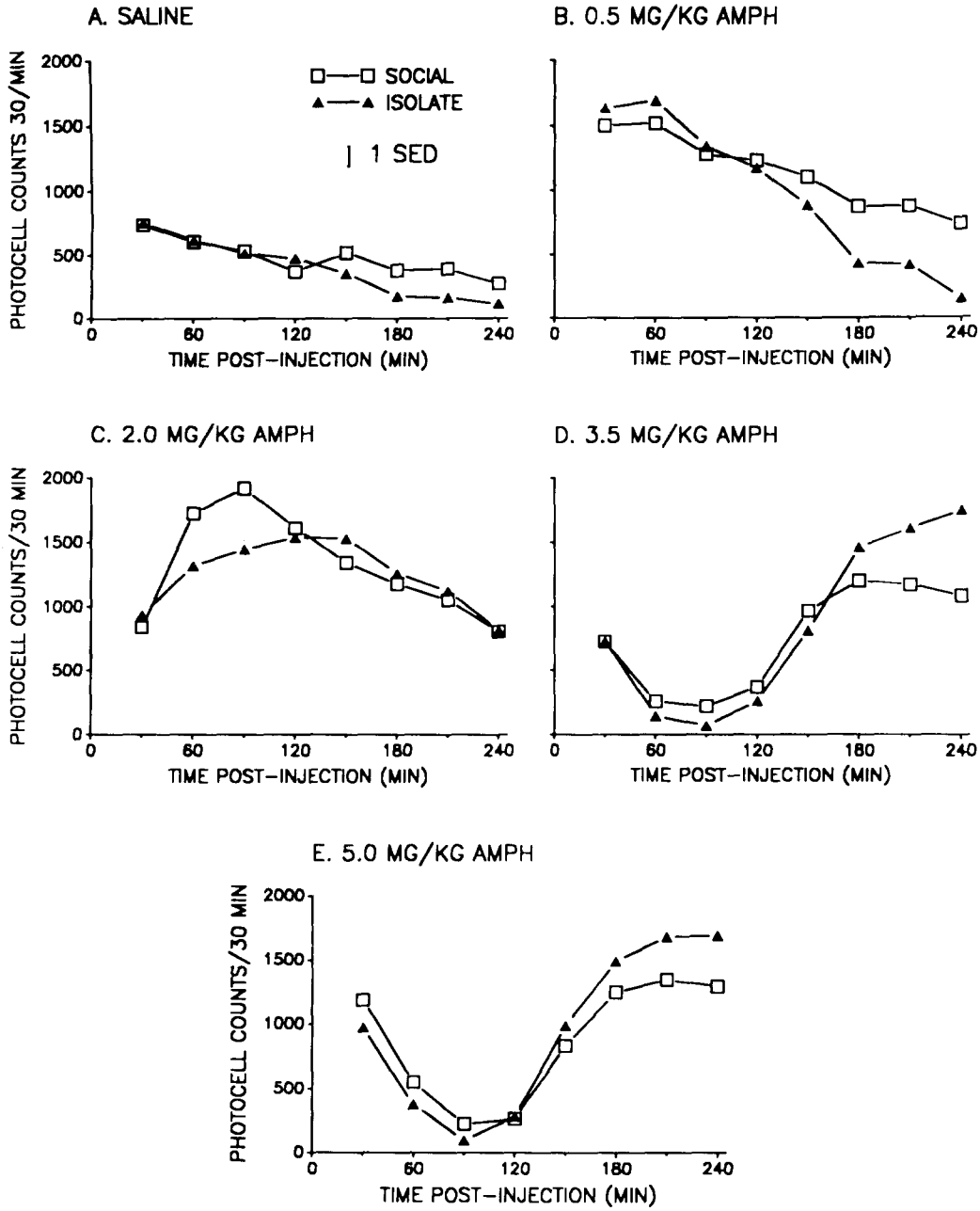

FIG. 1. Effects of isolation rearing on locomotor activity following *d*-amphetamine. The vertical bar represents the SE of the difference between means for the three-way interaction between rearing condition, dose, and time.

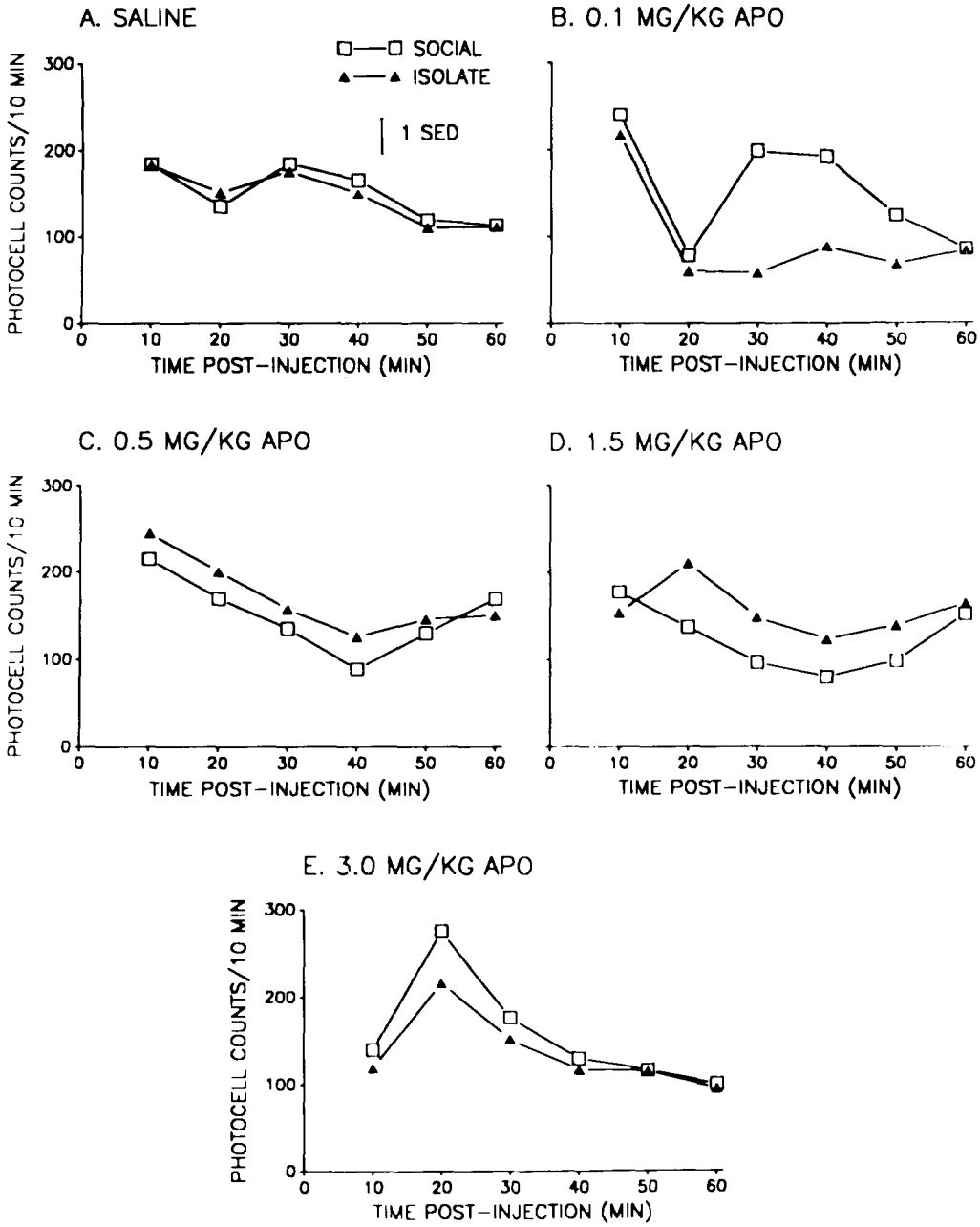

FIG. 2. Effects of isolation rearing on locomotor activity following apomorphine. The vertical bar represents the SE of the difference between means for the three-way interaction between rearing condition, dose, and time.

of apomorphine. Analysis of variance (ANOVA) did indicate a main effect of dose, $F(4, 56) = 3.62$, $p = 0.011$, mainly due to the reduction in activity following 0.1 mg/kg and the peak in activity at 20 min after 3.0 mg/kg, rearing × dose × time interaction, $F(20, 280) = 5.50$, $p < 0.001$.

There were clear dose-dependent effects of AMPH on behavioural stereotypy, with the most intense and prolonged response occurring at 5.0 mg/kg (Table 1). There were no significant effects of rearing condition on AMPH-induced stereotypy. However, there was a tendency for isolates to exhibit more intense stereotypy, particularly following the 5.0-mg/kg dose, the χ^2 value at 60 min being significant at the $p < 0.1$ level. There was a shift to the left in the peak in stereotypy intensity in isolated rats at the dose of 5.0 mg/kg. The isolates showed maximum stereotypy at 60 min, whereas the social controls peaked at 90 min; however, this difference did not reach significance, $F(3, 42) = 1.64$, n.s.

In marked contrast, isolation rearing significantly reduced the response to apomorphine. Isolated rats showed significantly less stereotypy following 1.5 and 3.0 mg/kg apomorphine only at those time points at which maximal stereotypy occurred (Table 1). For example, with the 1.5-mg/kg dose isolates showed significantly less intense stereotypy only at 30 min after injection. With the dose of 3.0 mg/kg, isolates

TABLE 1
BEHAVIOURAL STEREOTYPY FOLLOWING *d*-AMPHETAMINE OR APOMORPHINE

	χ^2	*df*	*p*	Mean Stereotypy Rating	
				Social	Isolate
d-amphetamine					
2.0 mg/kg					
60 min	1.99	2	n.s.	2.4	2.8
3.5 mg/kg					
30 min	1.53	2	n.s.	3.8	3.5
60 min	3.18	3	n.s.	4.4	4.3
90 min	3.20	3	n.s.	4.1	3.8
120 min	1.53	2	n.s.	3.5	3.4
5.0 mg/kg					
30 min	3.09	2	n.s.	3.3	3.8
60 min	4.87	2	n.s.	3.9	4.4
90 min	3.20	3	n.s.	4.4	4.1
120 min	3.09	3	n.s.	3.8	3.5
Apomorphine					
1.5 mg/kg					
30 min	6.64	3	<0.05	3.8	3.0
60 min	2.81	2	n.s.	2.4	1.8
3.0 mg/kg					
30 min	2.07	3	n.s.	4.3	3.8
60 min	8.66	3	<0.05	4.9	4.0

showed significantly less intense stereotypy only at 60 min, which was the time point for maximal stereotypy for this dose.

DISCUSSION

The present results indicate that isolation rearing in rats can alter both the locomotor activity and stereotypy induced by psychomotor stimulant drugs. Isolates showed an altered time course of AMPH-induced locomotor activity with an apparent shift to the left in the dose-response curve. Previous studies of the locomotor response to AMPH in isolation-reared rats have yielded conflicting results. Sahakian et al. (82) found no alteration in locomotor activity in isolation-reared rats at any dose of AMPH (0.5, 1.5, or 5.0 mg/kg). However, isolated rats were not only hyperactive following saline injection (80) but also immediately before drug injection (82), and the response to AMPH is known to be dependent upon baseline behaviour (19). Similar confounds with preinjection activity scores have also occurred in previous studies of the effects of social isolation (22,63).

Other studies have reported an enhancement of the locomotor response to AMPH in socially isolated rats (27,40). After long periods of isolation (10–12 months), isolated rats were spontaneously hyperactive and the difference in locomotor activity between isolates and controls increased threefold with a dose of 1.0 mg/kg AMPH (27).

The increased locomotor activity in isolates in the last hour of testing with 3.5 and 5.0 mg/kg AMPH corresponds with the fall in the AMPH-induced increase in extracellular concentration of DA [(110); see also Experiment 3] and with a reduction in the intensity of AMPH-induced stereotypy. These data suggest that optimum levels of extracellular DA are necessary to observe elevated locomotor activity in isolated rats.

The disruption of locomotor activity following apomorphine demonstrates the specificity of the effects of isolation. In isolated rats, 0.1 mg/kg apomorphine produced a more prolonged inhibition of locomotor activity. Apomorphine-induced hypoactivity following low doses of the drug (9,20) is thought to represent preferential stimulation of presynaptic D_2 autoreceptors (9,96). The second phase of the biphasic response to apomorphine presumably represents stimulation of postsynaptic DA receptors as it can be blocked by both D_1 and D_2 DA receptor antagonists (102). Thus, the present results suggest that either stimulation of presynaptic D_2 receptors has a greater effect in isolated rats or there is reduced sensitivity to the "second phase," the stimulation of postsynaptic receptors. Studies in mice have also reported an enhanced sedative effect of presynaptic doses of apomorphine in isolates (106). However, other studies of isolation in rats have not found alterations in the locomotor response to low doses of apomorphine (82), although the use of IP injections may have precluded finding results consistent with those of the present experiment. A significant rearing group × dose × time interaction was reported for 0.5 mg/kg apomorphine although no data are available for the precise temporal nature of this effect (80,82).

There is convincing evidence to suggest that hyperactivity induced by psychomotor stimulants is primarily mediated by DAergic mechanisms of the NAC (50). The present results therefore indicate possible alteration of DA-dependent function of the NAC following isolation rearing of rats. Indeed, isolates are more sensitive to the stimulating properties of AMPH infused directly into the NAC (40).

Isolated rats showed a tendency for increased stereotypy following AMPH while demonstrating a significantly reduced response to apomorphine. This response to AMPH is in agree-

ment with several previous studies (22,52,82). And, this effect is evident after isolation rearing (82) or social isolation of mature rats (52).

Previous studies have indicated an increase in apomorphine stereotypy following social isolation (82). However, the present results have not confirmed this effect. The reasons for this discrepancy are unknown. One possibility is that in the current study SC injections were used whereas previously apomorphine was administered IP, and the site of injection is known to influence the intensity of stereotyped behaviours following apomorphine (57). Sahakian et al. (82) only found enhanced stereotypy in isolated rats at 0.5 mg/kg apomorphine but not at either 0.1 or 1.5 mg/kg. This contrasts with the increase in stereotypy at virtually every dose of indirect DA agonists, including AMPH, cocaine, and pipradrol (82).

Taken together, these data suggest that presynaptic DA mechanisms are more consistently altered than postsynaptic processes by social isolation in rats. As both apomorphine- and AMPH-induced stereotypy are dependent upon the normal functioning of the CPu and the locomotor response to these drugs is largely determined by DAergic processes in the NAC (50), isolation-induced alteration in both these structures may be implicated by the present results. However, there is considerable competition for behavioural expression between these two structures (45,59). The interaction between stereotypy and locomotor activity is demonstrated by the fact that the attenuation of AMPH-induced stereotypy by CPu DA depletion produces an enhanced locomotor response to the drug (42,50). Furthermore, the preferential blockade of AMPH-induced locomotor activity by atypical neuroleptics, probably through their action on mesolimbic DA, can produce increased stereotyped responses to AMPH (77). Thus, alteration of either CPu or NAC DA function or both could produce changes in both the stereotypical and locomotor response to psychomotor stimulants.

These experiments have demonstrated that isolation rearing of rats can modify the locomotor and stereotypical responses to DA agonists. Sensitivity to the locomotor-stimulating properties of psychomotor stimulant drugs is known to undergo profound changes between infancy and adulthood in rats (55), and these experiments have demonstrated that the social environment can influence this development. Possible underlying neurochemical changes induced by isolation rearing are investigated in the following experiments.

EXPERIMENT 2

In this experiment, the postmortem levels of noradrenaline (NE), DA, and 5-hydroxytryptamine (5-HT) were determined simultaneously in a number of discrete brain regions from isolation-reared and socially reared rats. Tissue concentrations of the DA metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were also measured, together with the 5-HT metabolite, 5-hydroxyindoleacetic acid (5-HIAA).

Left and right sides of the brain were analyzed separately as DA function has been reported to be lateralised (31). Both groups of rats were experimentally naive and largely unhandled, as handling has been suggested to affect mesolimbic DA activity (23) and can influence brain laterality (18).

METHOD

Subjects

Subjects were 16 female Lister hooded rats obtained at 21 days of age. Eight were reared in social groups and eight were reared in isolation for 12 weeks, as described in Experiment 1.

Procedure

After 12 weeks of the differential rearing conditions, rats were killed by cervical dislocation and decapitation. Brains were rapidly removed and placed on an ice-cold plate. Olfactory bulbs were removed and the brain placed ventral surface uppermost in a dissection block. The PFC, NAC, aCPu, and pCPu were dissected as described previously (41) except the left and right sides of the brain collected separately. Tissue samples were stored at −70°C until assayed as described below.

High-Performance Liquid Chromatography Analysis

Brain tissue samples were weighed and homogenized in 0.5 ml 0.2 M perchloric acid, centrifuged, and the supernatant injected directly onto the high-performance liquid chromatography (HPLC) column. Chromatographic separation was accomplished using a stainless steel column (15 × 0.46 cm i.d.) packed with C_{18} reversed-phase, 5-μm Spherisorb ODS2 (Phase Separations Ltd., Clwyd, UK) fitted with a guard column (5 × 0.46 cm i.d.) containing the same packing material. The mobile phase consisted of 152 mM citric acid, 15 mM sodium acetate, 1.98 mM octane sulfonic acid, 0.8 mM EDTA, and 8% methanol and was adjusted to pH 3.6 with KOH (41). Detection was achieved using a BAS (West Lafayette, IN) LC-4B electrochemical detector. The glassy carbon working electrode was set at an applied potential of 0.71 V vs. an Ag/AgCl reference electrode.

Data Analysis

ANOVA (107) with one between-subjects factor, rearing condition, and two within-subject factors, brain area and side, was conducted for each compound of interest. Values for DA and DOPAC were subjected to a logarithmic transformation to achieve homogeneity of variance (107). All concentrations were calculated as ng/mg wet weight of tissue. The DOPAC/DA ratio was calculated as an index of DA activity (56). The HVA/DA and 5-HIAA/5-HT ratios were also calculated.

RESULTS

The results of the neurochemical analysis are shown in Tables 2 and 3. There were no significant effects of rearing condition on NE concentration in the medial PFC or NAC, $F(1, 7) = 0.81$, n.s. (Table 2), and NE levels did not differ between the two hemispheres, $F(1, 7) = 1.64$, n.s.

DA and Metabolites

The effects of isolation rearing on the concentration of DA in various brain regions are shown in Table 2. The concentration of DA in the PFC from isolates was 27% greater than in socially reared rats, rearing condition × area, $F(3, 21) = 5.59$, $p < 0.006$. Posthoc comparisons revealed that the effect of isolation on brain DA was specific to the PFC ($p < 0.01$). ANOVA also indicated a significant effect of side, $F(1, 7) = 17.90$, $p = 0.004$, which was independent of rearing condition and brain region, with the highest concentration on the right side of the brain.

Rearing conditions did not significantly affect the concentration of DOPAC, $F(1, 7) = 0.03$, n.s., in any brain area, $F(3, 21) = 2.15$, n.s. However, DOPAC concentrations also showed a marked asymmetry that differed between brain ar-

TABLE 2

EFFECT OF ISOLATION REARING ON BRAIN TISSUE CONCENTRATIONS OF NA, DA, DOPAC, AND HVA

	NA		DA		DOPAC		DOPAC/DA		HVA		HVA/DA	
	L	R	L	R	L	R	L	R	L	R	L	R
PFC												
Social												
$\bar{X}$	0.296	0.276	0.037	0.045	0.036	0.025	1.050	0.592	0.178	0.126	5.544	2.850
SEM	0.012	0.014	0.005	0.004	0.033	0.002	0.113	0.071	0.026	0.011	1.309	0.283
Isolate												
$\bar{X}$	0.301	0.300	0.052	0.052	0.041	0.029	0.824	0.573	0.138	0.134	2.829	2.661
SEM	0.018	0.011	0.005	0.003	0.004	0.002	0.079	0.045	0.013	0.009	0.353	0.354
NAC												
Social												
$\bar{X}$	0.479	0.579	6.691	8.237	2.294	2.748	0.343	0.337	0.589	0.770	0.088	0.095
SEM	0.132	0.128	0.413	0.505	0.138	0.164	0.015	0.018	0.035	0.094	0.004	0.011
Isolate												
$\bar{X}$	0.592	0.508	6.259	6.859	2.198	2.604	0.363	0.377	0.552	0.596	0.090	0.088
SEM	0.126	0.076	0.403	0.294	0.126	0.306	0.032	0.036	0.046	0.053	0.007	0.007
aCPu												
Social												
$\bar{X}$	n.d.		9.106	9.789	1.983	2.154	0.219	0.222	0.635	0.661	0.069	0.068
SEM			0.669	0.368	0.178	0.109	0.015	0.008	0.040	0.032	0.003	0.004
Isolate												
$\bar{X}$	n.d.		9.304	10.149	2.120	2.219	0.228	0.219	0.645	0.658	0.069	0.065
SEM			0.237	0.290	0.078	0.084	0.006	0.009	0.043	0.038	0.005	0.004
pCPu												
Social												
$\bar{X}$	n.d.		8.766	9.111	1.974	2.313	0.229	0.256	0.679	0.704	0.078	0.078
SEM			0.617	0.547	0.161	0.200	0.018	0.019	0.057	0.052	0.004	0.005
Isolate												
$\bar{X}$	n.d.		8.955	8.755	1.642	1.952	0.183	0.223	0.656	0.695	0.074	0.079
SEM			0.636	0.388	0.133	0.128	0.006	0.014	0.058	0.068	0.005	0.007

Concentrations expressed as ng/mg wet weight tissue. $n = 8$ for each rearing condition group.

eas, $F(3, 21) = 13.78$, $p < 0.001$. Posthoc comparisons showed that this bias in DOPAC concentration was only significant in the PFC ($p < 0.01$), with the right side being approximately 30% lower than the left side of the brain.

The concentration of HVA was not significantly affected by rearing condition, $F(1, 7) = 0.92$, n.s., nor did it differ between left and right sides of the brain, $F(1, 7) = 0.59$, n.s.

The DOPAC/DA ratio is shown in Table 2. Rearing rats in isolation did not significantly affect the ratio of DOPAC/DA concentration in any brain region, $F(1, 7) = 1.49$, n.s. A significant area × side interaction, $F(1, 7) = 9.53$, $p < 0.001$, indicated that an asymmetry in DOPAC/DA ratio was dependent upon brain structure. Posthoc comparisons revealed that this ratio was asymmetric in the PFC ($p < 0.01$), with this measure of DAergic activity being greatest in the left hemisphere. There was also a trend for the asymmetry to be less in the PFC from isolated reared than in controls (Fig. 3).

There was no main effect of rearing condition on the HVA/DA ratio, $F(1, 7) = 1.92$, n.s., and the rearing × area interaction did not reach significance, $F(3, 21) = 2.68$, $p = 0.073$. In contrast to the results for the DOPAC/DA ratio, there were no overall effects of side, $F(1, 7) = 1.74$, n.s., and no side × area interaction, $F(3, 21) = 1.77$, n.s. However, there was a significant three-way interaction between side, area, and rearing condition, $F(3, 20) = 4.14$, $p = 0.020$. In socially reared rats, there was a marked asymmetry in the HVA/DA ratio in the PFC ($p < 0.01$), this ratio being approximately 100% greater in the left hemisphere than in the right. This bias in DA activity was not evident in isolation-reared rats, which had equivalent levels on each side of the brain that were similar in magnitude to the concentration in the right side of controls (Fig. 3).

5-HT and 5-HIAA

Rearing conditions did not affect the brain levels of 5-HT in any structure, $F(1, 7) = 1.13$, n.s. A significant main effect of side, $F(1, 7) = 7.44$, $p = 0.029$, indicated that the levels of 5-HT were greater in the right side of the brain, mainly in subcortical structures (Table 3).

The tissue concentration of 5-HIAA is also shown in Table 3. There were no significant effects either of rearing condition, $F(1, 7) = 0.10$, n.s., or side, $F(1, 7) = 1.29$, n.s.

Rearing in isolation produced a significant change in the 5-HIAA/5-HT ratio, rearing × area, $F(3, 20) = 3.56$, $p = 0.033$. Posthoc comparisons revealed that this ratio was 18% lower in the NAC from isolates ($p < 0.05$) with no effects in any other brain region. ANOVA indicated that there were no hemisphere differences in this ratio, $F(1, 7) = 0.72$, n.s., in any brain region, $F(3, 21) = 0.18$, n.s.

DISCUSSION

Rearing rats in isolation produced specific and relatively large changes in the postmortem tissue concentration of

TABLE 3

EFFECT OF ISOLATION REARING ON BRAIN TISSUE CONCENTRATIONS OF 5-HT AND 5-HIAA

	5-HT		5-HIAA		5-HIAA/5-HT	
	L	R	L	R	L	R
PFC						
Social						
$\bar{X}$	0.525	0.507	0.311	0.330	0.592	0.658
SEM	0.014	0.026	0.018	0.013	0.030	0.028
Isolate						
$\bar{X}$	0.505	0.531	0.375	0.358	0.750	0.674
SEM	0.020	0.006	0.014	0.016	0.039	0.034
NAC						
Social						
$\bar{X}$	0.559	0.685	0.777	0.864	1.461	1.354
SEM	0.051	0.075	0.024	0.082	0.118	0.109
Isolate						
$\bar{X}$	0.363	0.377	0.744	0.751	1.181	1.130
SEM	0.032	0.036	0.046	0.050	0.119	0.140
aCPu						
Social						
$\bar{X}$	0.219	0.222	0.448	0.461	0.989	0.912
SEM	0.015	0.008	0.012	0.040	0.063	0.061
Isolate						
$\bar{X}$	0.228	0.219	0.466	0.504	0.994	0.995
SEM	0.006	0.009	0.017	0.020	0.063	0.050
pCPu						
Social						
$\bar{X}$	0.229	0.256	0.489	0.520	0.950	0.920
SEM	0.018	0.019	0.025	0.036	0.034	0.041
Isolate						
$\bar{X}$	0.183	0.223	0.512	0.531	0.893	0.973
SEM	0.006	0.014	0.026	0.030	0.042	0.027

Concentrations expressed as ng/mg wet weight tissue. $n = 8$ for each rearing condition group.

monoamines and their metabolites. Isolated rats had significantly higher concentrations of DA (+27%) in the medial prefrontal cortex when compared to socially reared controls, but no alteration in the NAC or CPu. In contrast, the tissue concentration of NE in the PFC was unaffected by rearing conditions. Selective alterations in cortical DA function have been reported previously following social isolation in rats (5,38). In one study (5), socially isolated rats had a lower DOPAC/DA ratio in the PFC than group-housed controls, a difference that could be reversed by resocialisation. As in the present study, this alteration in PFC DA was also specific in nature, as there were no significant changes in the NAC or CPu (5). The reduction in this measure of DA activity (56) was mainly due to a decrease in DOPAC levels, although there was also a trend for PFC DA to be higher in isolated rats (5). The present results also showed a reduction in DOPAC/DA ratio in isolated rats, and although it did not reach significance it was of a similar magnitude to that reported by Blanc et al. (5). The significant increase in PFC DA following isolation in the present experiment, and tendencies in other experiments (5,73), also indicate a reduction in DAergic activity in this region, as increased DAergic activity in the PFC occurs in parallel with significantly lower levels of tissue DA (56).

It is worth pointing out that the present study examined the effects of isolation rearing whereas the previous report indicating reduced DA activity in the PFC in isolated rats used mature subjects housed in isolation (5). These procedural differences are known to influence the sequelae of social isolation (21,44,86). The general agreement between the present results and those of previous studies (5) indicates that the effects of isolation on PFC DA activity are not specific to rearing conditions but may be a consequence of social isolation per se.

There is also other evidence for an alteration in PFC DA following social isolation (70). After 1 year of isolation, PFC neuronal sensitivity to iontophoretically applied DA was increased, although this effect was not apparent after 3 months of isolation (70). This would suggest that the increased neuronal sensitivity to DA could be a response to reductions in DA turnover in this region.

Although the basal turnover of PFC DA is reduced in isolates, following foot-shock stress the PFC DOPAC/DA ratio increased to a level greater than in stressed controls (5). This increased responsiveness of mesocortical DA to foot-shock in isolates may reflect reduced feedback control in this structure (108) and in general terms is another example of enhanced reactivity following social isolation.

Early experience is known to be able to influence brain

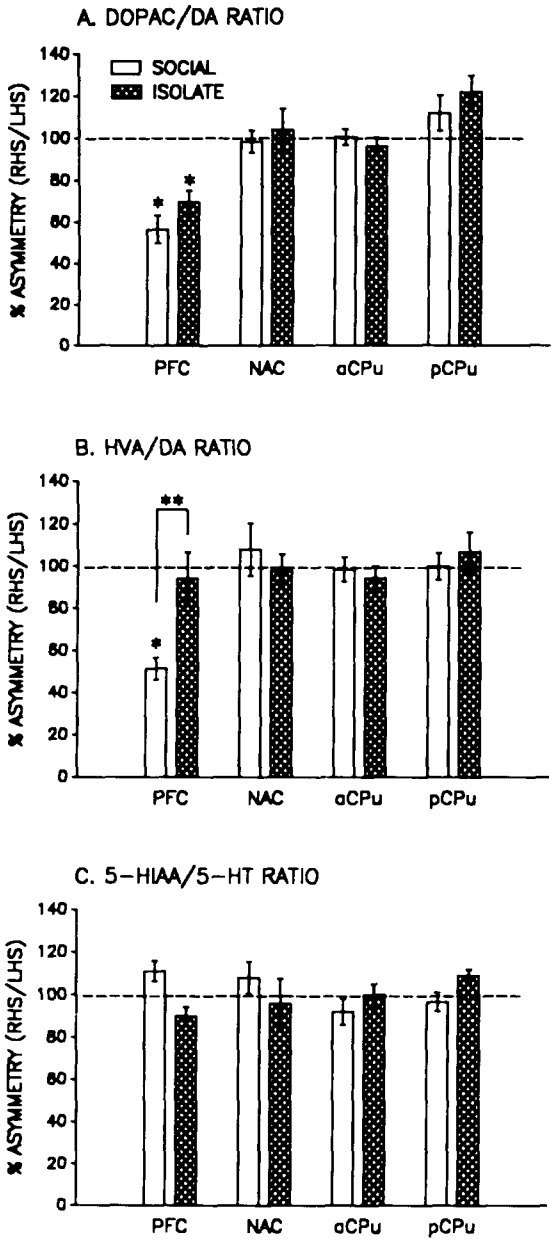

FIG. 3. Effects of isolation rearing on asymmetry in metabolite/monoamine ratios. Values shown are the ratio in the right-hand side (RHS) expressed as a percentage of the value in the left-hand side (LHS). The broken line represents no asymmetry. (A) DOPAC/DA ratio. (B) HVA/DA ratio. (C) 5-HIAA/5-HT ratio. *p compared with no asymmetry. **$p < 0.01$ compared with asymmetry in socially reared controls.

laterality in later life (18) and the asymmetry in PFC DA metabolism was also influenced by rearing experience, as the asymmetry in DA metabolite/DA ratios were less pronounced in the PFC from isolated rats. The functional consequences of this nonpharmacologically invoked alteration in PFC DA asymmetry are unknown, although isolated rats do show greater rotational movements when exposed to a novel environment (30). Both unilateral DA-depleting lesions of the PFC (78) and infusions of DAergic drugs into this structure can influence behavioural lateralisation (92).

In agreement with previous reports (31,39), a marked asymmetry in monoamine function was evident in the present study. DA concentrations were higher in the right side of the brain. Tissue concentrations of DOPAC, 5-HT, and the DOPAC/DA and HVA/DA ratios also showed marked asymmetries. These effects are unlikely to be due to differences in dissection for the two sides of the brain, as no asymmetry was evident in NA, 5-HIAA or 5-HIAA/5-HT ratio measured in the same tissue samples. Therefore, these results demonstrate a significant difference in monoamine concentration between hemispheres in the rat. DA asymmetries in rats have now been widely reported and have been correlated with behavioral measures, such as the preferred direction of rotation (109), spontaneous side preference in lever pressing (31), Y-maze side preference (15), and drug-induced circling (39). Asymmetry in 5-HT metabolism has also been reported (109).

Isolation rearing also produced a significant reduction in 5-HT activity that was specific to the NAC, the 5-HIAA/5-HT ratio in the NAC being reduced by 18% in isolated rats. Changes in 5-HT metabolism (69,93) and reductions in septal tryptophan hydroxylase activity have also been reported in isolated rats (88).

The present results add to a growing body of evidence that 5-HT activity is reduced following social isolation. Moreover, this experiment has demonstrated that reduction in 5-HT activity is specific to certain brain areas. For example, this ratio was not altered in the aCPu or pCPu. The apparent reduction in NAC 5-HT activity in isolates may contribute to isolation-induced hyperreactivity as 5-HT can modulate DA activity in this structure and rats with either lesions of the raphe or with 5-HT-depleting lesions of the NAC are also hyperreactive (91).

EXPERIMENT 3

Recently, an intracerebral dialysis technique has been developed to measure changes in extracellular amines and their metabolites that involves implantation of a small length of dialysis membrane into discrete brain regions (101,110). Intracerebral dialysis is now widely used for the sampling of many neurotransmitters, including DA (110,111), and provides a method for in vivo determination of neurochemical function.

The extracellular levels of DA, DOPAC, and HVA, together with the 5-HT metabolite 5-HIAA, were determined in vivo in both socially reared and isolation-reared rats using intracerebral dialysis. The response to AMPH was measured in both the CPu and NAC. As previous studies have indicated time-dependent effects of isolation on neurotransmitter function [e.g., (70)], this response was assessed after two periods of isolation. To allow direct comparison with the results of the first two experiments and previous behavioural studies (40,42,44,82), isolation-reared rats were compared to socially reared controls at 3–4 months postweaning. In addition, isolates and social controls were compared after 9 months of differential rearing conditions to allow comparisons with other neurochemical studies on the effects of social isolation that used similar time periods (5,27,33).

METHOD

Subjects

Subjects were 46 female Lister hooded rats. Twenty-three were reared from weaning age in social groups and 23 were reared in isolation, as described in Experiment 1.

Procedure

Dialysis probes were prepared as previously described (110) from cellulose dialysis tubing (200 μm o.d.; Hemoflow C1,3, Fresenius AG, Hamburg, Germany). Two- and 5-mm length probes were used for the NAC and CPu, respectively.

After either 3–4 or 9 months of the differential rearing conditions, subjects were lightly anaesthetised by placing them in a jar containing halothane and then in a stereotaxic frame with an anaesthesia chamber attached. Anaesthesia was maintained throughout the experiment by administering 0.8% halothane in oxygen, a procedure known to have little effect on DAergic activity (26). A negative pressure was maintained in the anaesthetic chamber to prevent the anaesthetic mixture from escaping and to permit the introduction of air from around the animals' heads. Core body temperature was maintained at 37° C with the aid of a rectal thermometer and a heated blanket. The dialysis probes were secured in place using skull screws and dental cement. Stereotaxic coordinates for the NAC were: AP = +3.4 mm, L = 1.7 mm, V = −7.5 mm from dura; and for the CPu: AP = +2.2 mm, L = −3.2 mm, V = −6.0 mm from dura, with the incisor bar set at +5.0 mm (74).

The dialysis probes were perfused with Ringers' saline containing: 147.0 mM NaCl, 4.0 mM, KCl and 3.4 mM $CaCl_2$. The pH was adjusted to 6.5 using 0.05 M NaOH (105). A Harvard microinfusion pump (Harvard Instruments Ltd., South Natick, MA) delivered the perfusate at 2 μl/min via polyethylene tubing. The perfusate was collected for 30 min in small Eppendorf tubes containing 10 μl 1 M PCA and immediately injected onto the HPLC column and assayed for DA, DOPAC, HVA, and 5-HIAA. A minimum of four samples were collected before AMPH administration. AMPH was prepared in a concentration of 4 mg/ml in 0.9% saline and injected 0.5 ml/100 g body weight (2 mg/kg body weight). As a control for the nonspecific effects of injection, two rats from each rearing condition group were administered saline. All injections were SC in the back of the neck. Following drug treatment, perfusate samples were collected for 4 h.

At the completion of the testing, subjects were administered an overdose of barbiturate and perfused transcardially with saline followed by 10% formalin. Following fixation, coronal sections (60 μm) were cut on a freezing microtome and every third section through the appropriate structure was mounted and stained with cresyl violet for the determination of cannulae placements.

Analysis of Perfusates

Perfusates were immediately injected onto the HPLC column. Perfusate monoamine concentrations were quantified by comparison with standard solutions containing known concentrations. Analysis of the dialysates was conducted using two different sets of HPLC parameters to provide additional information about the specificity of the peaks detected. One of the analysis methods used the conditions described in Experiment 2. The other used a mobile phase consisting of: 130 mM monochloroacetic acid, 0.4 mM heptane sulphonic acid, 0.1 mM EDTA, and 10% HPLC grade methanol. The pH was adjusted to 3.0 with KOH. The compounds of interest were detected using electrochemical detection with a glassy carbon working electrode. The applied voltage was maintained at 0.65 V vs. an Ag/AgCl reference electrode.

The two sets of HPLC parameters produced two different elution orders for the compounds of interest. For the monochloroacetic-acid-based mobile phase, the order of elution was: DA, DOPAC, 5-HT, 5-HIAA, and HVA. The elution order for the sodium acetate based mobile phase was: DOPAC, DA, 5-HIAA, HVA, and 5-HT. The limit of detection for the monoamines was approximately 0.04 pmol.

RESULTS

Basal Perfusate Concentrations

Histological assessment of brains revealed that the majority of dialysis probes were placed in the intended structure. Any subject with an inappropriate placement was excluded from the experiment. The initial high levels of DA (presumably due to neuronal damage) were allowed to fall before measurements of basal concentration were taken. The implantation of dialysis probes into the CPu and NAC permitted the measurement of basal levels of DA, DOPAC, HVA, and 5-HIAA (Tables 4 and 5). NE and 5-HT were not routinely detectable in the perfusates. Following injection of saline, there were no measurable changes in the concentration of any compound and therefore these results will not be discussed further.

Rearing rats in isolation produced relatively large and regionally specific effects on the extracellular concentration of the four compounds. In the NAC, there were no rearing condition effects on the concentration of DA, $F(1, 15) = 0.40$, n.s., DOPAC, $F(1, 15) = 0.16$, n.s., or HVA, $F(1, 15) = 1.12$, n.s. There was, however, a significant effect of rearing

TABLE 4

PERFUSATE CONCENTRATIONS OF NUCLEUS ACCUMBENS DA, DOPAC, HVA, AND 5-HIAA PRIOR TO ADMINISTRATION OF *d*-AMPHETAMINE

	pmol/60 μl Perfusate			
	DA	DOPAC	HVA	5-HIAA
3 Months				
Social (n = 5)	0.22 ± 0.06	33.99 ± 0.52	20.20 ± 3.35	14.50 ± 1.64
Isolate (n = 6)	0.36 ± 0.12	38.41 ± 9.97	21.20 ± 4.92	8.47 ± 0.65
9 Months				
Social (n = 3)	0.19 ± 0.03	13.40 ± 1.72	12.97 ± 2.33	13.31 ± 1.36
Isolate (n = 4)	0.12 ± 0.04	16.72 ± 3.40	10.83 ± 1.60	9.31 ± 0.68

Each value is the mean ± SEM (number of subjects is shown in parentheses) of the three perfusate samples collected immediately prior to administration of *d*-amphetamine.

TABLE 5

PERFUSATE CONCENTRATIONS OF CAUDATE PUTAMEN DA, DOPAC, HVA, and 5-HIAA PRIOR TO ADMINISTRATION OF *d*-AMPHETAMINE

	pmol/60 μl Perfusate			
	DA	DOPAC	HVA	5-HIAA
3 Months				
Social (n = 5)	0.87 ± 0.51	46.55 ± 5.46	29.32 ± 4.31	8.63 ± 1.17
Isolate (n = 5)	0.11 ± 0.03	76.50 ± 17.76	44.89 ± 3.14	7.83 ± 1.33
9 Months				
Social (n = 6)	0.19 ± 0.03	26.74 ± 3.09	21.40 ± 1.45	10.40 ± 1.47
Isolate (n = 6)	0.15 ± 0.03	34.98 ± 3.04	25.32 ± 2.38	9.32 ± 1.76

Each value is the mean ± SEM (number of subjects is shown in parentheses) of the three perfusate samples collected immediately prior to administration of *d*-amphetamine.

on the basal concentration of the 5-HT metabolite, 5-HIAA (Table 4). Isolated rats had a greatly reduced level of 5-HIAA at both 3 and 9 months after weaning, $F(1, 15) = 22.10$, $p < 0.001$. The 5-HIAA concentration in isolates at 3 months was 42% lower than in socially reared controls and at 9 months it was 37% lower.

There were also significant age-related alterations in the basal concentrations of the monoamines measured in the NAC. There was a small, nonsignificant reduction in DA concentration in the older groups of rats, $F(1, 15) = 2.47$, n.s., and large reductions in the concentration of both DOPAC, $F(1, 19) = 5.77$, $p < 0.05$, and HVA, $F(1, 15) = 5.15$, $p < 0.05$. These age-related reductions in DA metabolite levels were approximately 50%. There were no differences in NAC 5-HIAA concentration between the 3- and 9-month groups, $F(1, 15) = 0.03$, n.s., indicating the specific nature of these age-related changes in neurotransmitter function.

In the CPu, isolated rats did not differ from social controls in the concentration of 5-HIAA at either time period, $F(1, 19) = 1.12$, n.s. (Table 5). The CPu perfusate concentration of DA did not differ between the two rearing condition groups, $F(1, 19) = 2.71$, n.s.; however, isolation rearing did affect the concentration of DOPAC and HVA, with both compounds being present in higher concentration in the perfusates from isolates. This effect of isolation on DOPAC and HVA was present at both 3 and 9 months, although it only reached significance for DOPAC. These isolation-induced increases were approximately 50% for DOPAC, $F(1, 19) = 4.59$, $p < 0.05$, and 37% for HVA, $F(1, 19) = 3.60$, n.s.

Age-related changes in DA function were also evident in CPu perfusates. DA was unaffected, $F(1, 19) = 2.02$, n.s., but as in the NAC there were reductions in both DOPAC, $F(1, 19) = 13.05$, $p < 0.01$, and HVA, $F(1, 19) = 7.93$, $p < 0.025$, in the 9-month groups. There were no differences in CPu 5-HIAA concentration with age. None of the age-related changes in perfusate monoamine concentration were significantly affected by rearing conditions.

Effects of AMPH Administration

Administration of 2 mg/kg AMPH increased the concentration of DA in the perfusate in all rats in both the NAC and CPu (Fig. 4). The largest increases in DA were in the CPu, as indicated by a main effect of area, $F(1, 32) = 43.79$, $p < 0.001$. ANOVA also indicated a significant main effect of rearing condition on the levels of DA following AMPH, $F(1, 32) = 6.54$, $p = 0.016$, with isolated rats showing greater increases than socially reared rats. A nonsignificant interaction of rearing condition × area, $F(1, 32) = 2.84$, n.s., also indicated that this effect of isolation on DA levels did not differ significantly between the CPu and NAC. Perfusate DA concentration peaked in all groups in the second sample collected, indicating a maximal release between 30 and 60 min, consistent with the time course of the behavioural effects of the drug (42). There was also a tendency for isolated rats to show a prolonged elevation in DA levels following AMPH, rearing condition × time, $F(7, 209) = 2.00$, $p = 0.057$.

The age of the subject also affected the time course of extracellular DA levels, $F(7, 209) = 7.97$, $p < 0.001$, with the 9-month groups showing a later peak in maximal concentration that was not significantly affected by rearing condition, $F(7, 209) = 1.17$, n.s.

In agreement with other studies [e.g., (110)], the extracellular concentration of DOPAC fell markedly following AMPH administration (Fig. 5), with the maximum reduction occurring 90–120 min after injection. This decrease in DOPAC after AMPH administration was not only greater overall in the CPu than in the NAC, $F(1, 33) = 21.04$, $p < 0.001$, but also showed slower recovery, $F(7, 221) = 17.96$, $p < 0.001$. Consistent with the greater elevation of extracellular DA following AMPH in isolates, the perfusate concentration of DOPAC was reduced to the greatest extent in isolation-reared rats, $F(1, 33) = 7.99$, $p = 0.008$. A significant interaction between rearing × area, $F(1, 33) = 5.03$, $p = 0.032$, indicated that the effect of isolation on DOPAC levels after AMPH was greatest in the CPu. Isolated rats also showed a slower recovery from the maximum reduction in DOPAC concentration, $F(7, 221) = 2.33$, $p = 0.026$, which again was more evident in the CPu, $F(7, 221) = 4.43$, $p < 0.001$.

There was also a tendency for older rats to show a slower recovery from the decrease in DOPAC concentration produced by AMPH administration, $F(7, 221) = 1.91$, $p = 0.07$. A significant three-way interaction between rearing, age, and time, $F(7, 221) = 2.52$, $p = 0.016$, indicates that the effects of isolation differ between the two time periods.

HVA also showed a marked reduction following AMPH; however, in contrast to DOPAC, the fall in HVA level did not differ between CPu and NAC, $F(1, 33) = 3.16$, n.s., and was not influenced by rearing condition, $F(1, 33) = 2.11$, n.s. The recovery from the reduction in HVA following AMPH was, however, slower in the older group of rats, $F(7, 224) = 2.36$, $p = 0.024$. As with DOPAC concentrations, this effect of aging was amplified by isolation rearing, $F(7, 224) = 4.39$, $p < 0.001$. A significant three-way interaction between rear-

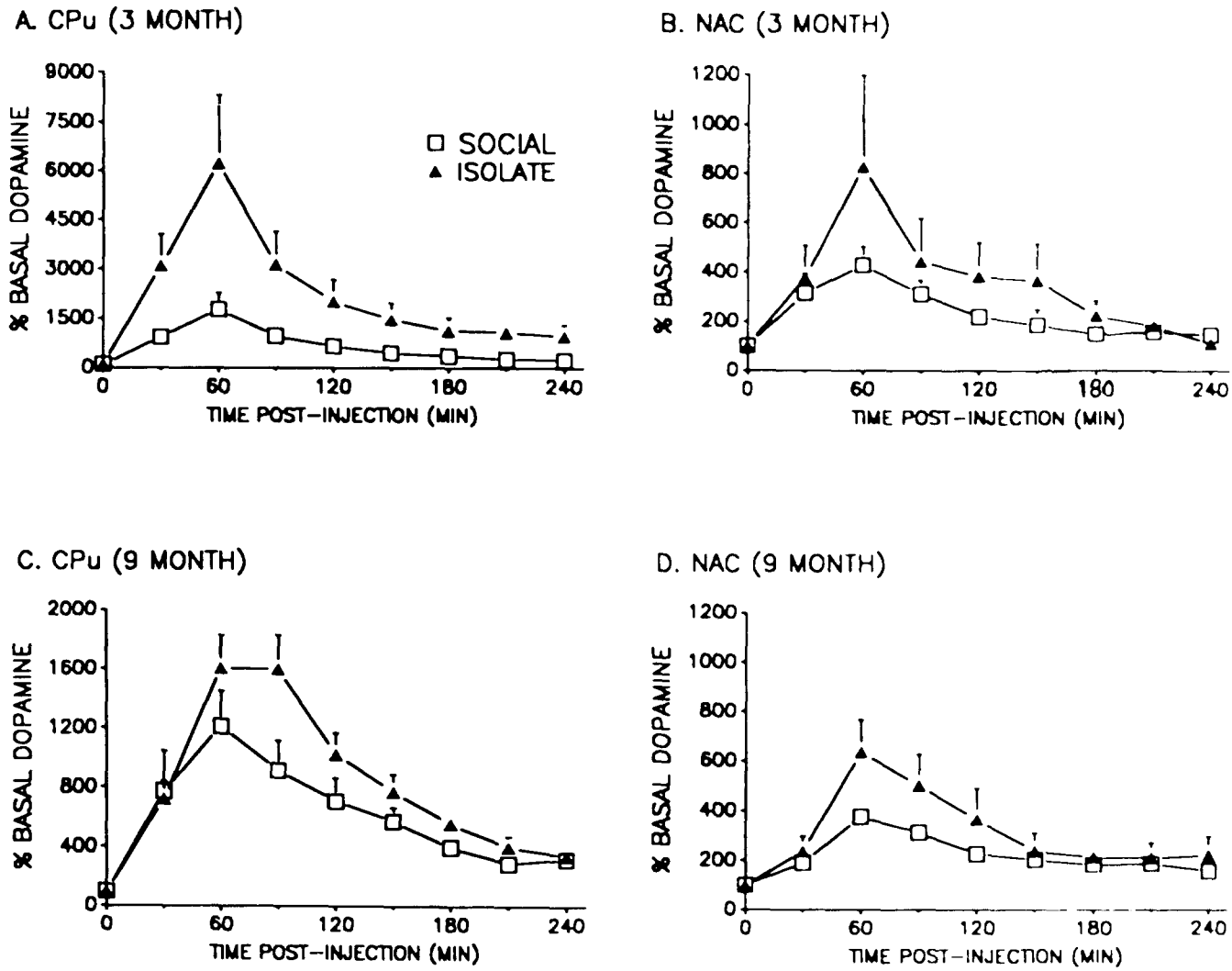

FIG. 4. Effects of isolation rearing on extracellular concentration of dopamine (% of basal value) in dialysis perfusates from the caudate putamen (CPu) and nucleus accumbens (NAC) following 2 mg/kg *d*-amphetamine SC. The basal value was the mean of the three samples collected immediately prior to *d*-amphetamine administration. Vertical bars represent the SEM. (A and B) After 3 months of isolation. (C and D) After 9 months of isolation.

ing, area, and time, $F(7, 224) = 6.32$, $p < 0.001$, indicates that the effects of isolation on the time course HVA levels differed between the NAC and CPu. The concentration of perfusate HVA from the NAC fell faster in isolated rats than in socials, whereas in the CPu reduced perfusate levels in isolated rats were seen only in later samples.

In marked contrast to the effects of AMPH on the extracellular concentration of DA and its metabolites, there were no significant effects on the perfusate concentration of the 5-HT metabolite, 5-HIAA. There were also no significant effects either of rearing condition, $F(1, 32) = 0.06$, n.s., or of brain region, $F(1, 32) = 3.35$, n.s.

DISCUSSION

Experiment 3 demonstrated in vivo that environmental conditions during early development can alter the extracellular concentrations of monoamines and metabolites and the dopaminergic response to AMPH.

Basal extracellular concentrations of DA were not significantly affected by rearing condition either in the CPu or NAC. However, the concentrations of both DOPAC and HVA were increased in CPu perfusates from isolates. The reasons for this are unclear. One possibility is increased DA uptake (9) and metabolism or increases in synthesis and release. Synthesis and release of CPu DA is under regulation from presynaptic D_2 receptors (9,95), and one explanation of these results is that they are due to alterations in autoreceptor feedback control. In support of this view, isolated rats show a reduced frequency of penile erections and stretching and yawning in response to autoreceptor-activating doses of DA agonists (24), possibly indicating reduced presynaptic sensitivity. However, the results of Experiment 1, indicating enhanced apomorphine-induced hypoactivity in isolates and therefore possibly increased autoreceptor sensitivity, would seem not to support such an effect. Another possibility is an increase in the spontaneous activity of nigral neurons stimulated via the postsynaptic neuronal feedback loop (7).

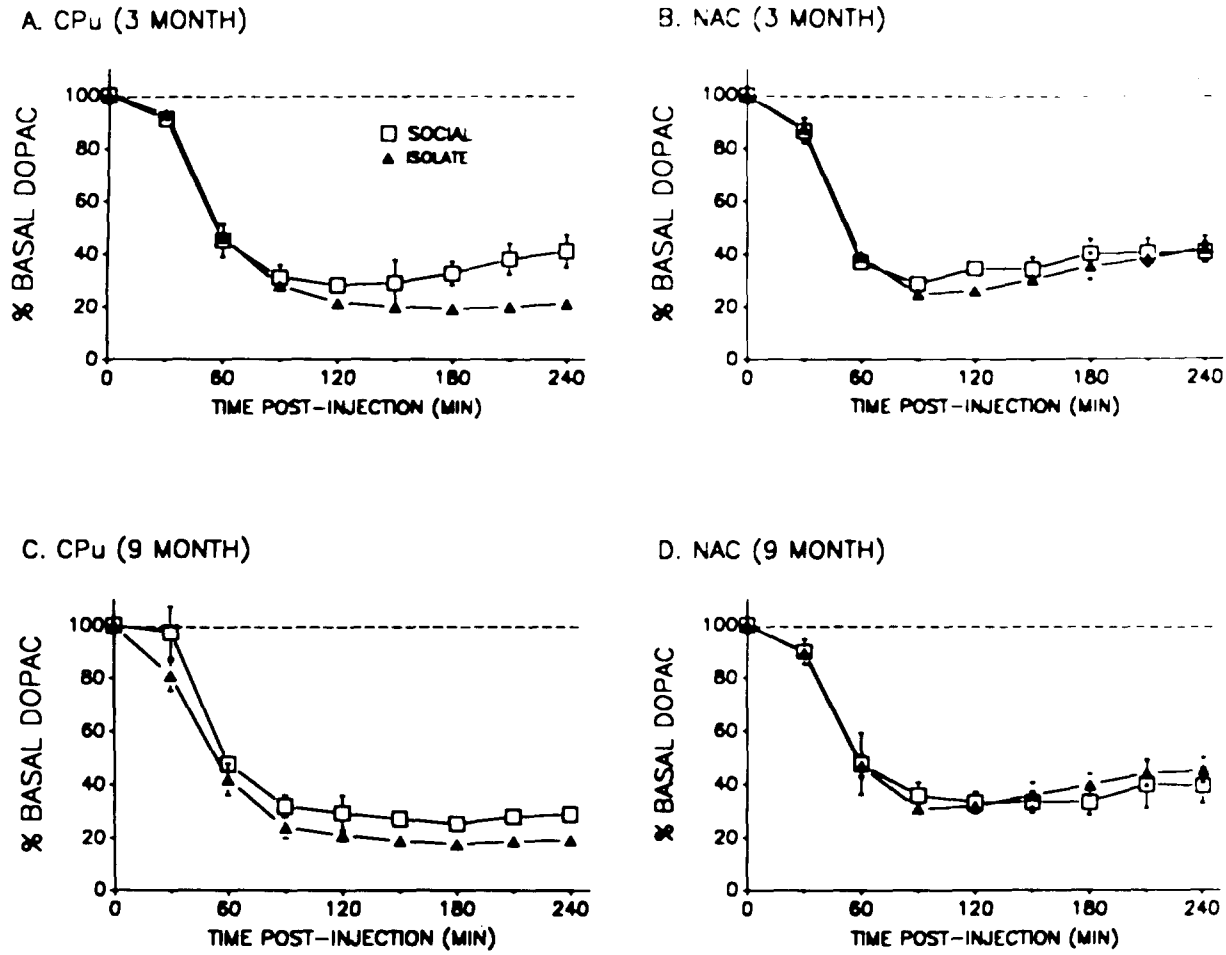

FIG. 5. Effects of isolation rearing on extracellular concentration of DOPAC (% of basal value) in perfusates from the caudate putamen (CPu) and nucleus accumbens (NAC) following 2 mg/kg *d*-amphetamine SC. The basal value was the mean of the three samples collected immediately prior to *d*-amphetamine administration. Vertical bars represent the SEM. (A and B) After 3 months of isolation. (C and D) After 9 months of isolation.

In NAC perfusates from isolated rats, there were no changes in basal levels of DA, DOPAC, or HVA, but large and reproducible decreases in 5-HIAA concentration were evident. This appears to confirm the reduction in the 5-HIAA/5-HT ratio seen postmortem in the NAC from isolates in Experiment 2 and provides further evidence for a serotonergic involvement in the effects of isolation rearing (41,69). As DA activity in the NAC can be modulated by NAC 5-HT activity (43), this reduction in 5-HT activity could contribute to altered DA function and increased locomotor activity in isolates.

Following AMPH administration, isolated rats showed an enhanced release of DA compared to socially reared controls, this effect being greater in the CPu than in the NAC. This increase in DA release may result from an increase in the AMPH-sensitive DA pool (85), and reports of an increase in postmortem levels of DA in the CPu from isolates would support such a view (104), although Experiment 2 did not confirm these findings. A fall of nearly 20% in the levels of CPu DA in isolates on exposure to an open field, an effect not observed in controls, presumably indicates greater release than synthesis (104).

An alternative explanation is that the present results may reflect metabolic changes in isolates (e.g., increased permeability of the blood-brain barrier, differences in cerebral blood flow, or changes in uptake mechanisms). For example, cortical capillary volume (4) and the number of mitochondria per neuron (89) are reduced in isolated rats.

Isolated rats also showed a greater reduction in DOPAC concentration following AMPH, particularly in the CPu. This decrease in either extracellular (110) or tissue levels of DOPAC (53) following systemic AMPH is thought to reflect local action of the drug within the CPu (111). The striatonigral feedback loop is probably of little importance in this metabolite response to AMPH (111), indicating that the greater reduction of DOPAC in isolated rats may represent alteration in local function.

The lack of reliable differences between rearing condition groups in the HVA response to AMPH suggests there might be greater inhibition of monoamine oxidase (MAO) by AMPH in isolated rats. Whereas HVA is derived from both DOPAC, and from the metabolism of 3-methoxytyramine (3-MT) by catechol-*O*-methyl-transferase, DOPAC is formed from the direct interneuronal metabolism of DA by MAO.

Enhanced uptake mechanisms in isolates may also contrib-

ute to the differences in basal concentrations and AMPH response. Greater uptake of DA, and therefore increased substrate for MAO, would explain enhanced DOPAC levels under basal conditions and could also lead to increased intraneuronal levels of AMPH. This could lead to increased DA release and decreased DOPAC, as it has been suggested that AMPH-induced reductions in DOPAC are due to the switching of newly synthesised DA away from MAO and into the syanpse (111).

Age-Related Changes

Another important finding is marked differences in DAergic markers between the 3- and 9-month groups that were independent of rearing condition. Basal levels of both DOPAC and HVA were reduced in the CPu and NAC perfusates from both 9-month groups, although DA levels were not affected. There are now numerous reports of age-related alterations in DAergic mechanisms, with a general agreement of reduced activity with age (100). DA-sensitive adenylate cyclase activity, DA receptor number, and DA release have all been shown to reduce with age (79,100).

The present findings are consistent with previous studies. K^+-evoked release of DA, measured with in vivo electrochemistry, is significantly reduced with age, and in agreement with the current experiment the time course of release was significantly prolonged (79). The present results show that the peak in AMPH-induced DA release and the fall in DOPAC were longer lasting in older rats, and it is a general finding that the capacity of DA systems to adapt to change declines with age (100).

There were no interactions between age and rearing condition, although there were trends for the effects of isolation to reduce with duration. This finding also agrees with previously published studies. Isolation-induced increases in the intensity of apomorphine stereotypy (13) and the sensitivity of striatal neurons to centrally applied DA (70) reduce with increased isolation periods.

The results of this experiment demonstrated that isolation rearing can affect presynaptic DA function in the CPu and NAC. Whether the effects of isolation on CPu function are exclusive to DA terminals or are also present postsynaptically is examined in Experiment 4.

EXPERIMENT 4

Several studies have implicated alterations in CPu DA receptor mechanisms in the behavioural effects of social isolation [e.g., (52,82)]. Most reports have inferred alterations in DA receptor function from measuring the behavioral responses to pharmacological agents such as AMPH (104) or apomorphine (17). A few studies have directly measured DA receptor function following social isolation of rodents but with contradictory findings (33,106).

In the mammalian CNS, DA is now thought to act at least three receptor subtypes (16,87,90). These different classes of DA receptors have been characterised as D_1, D_2, and D_3 on the basis of their pharmacological properties and association with the enzyme adenylate cyclase (46,82,90). Activation of D_1 receptors has been shown to stimulate adenylate cyclase activity and the production of cyclic AMP, whereas activation of at least some D_2 receptors is thought to inhibit adenylate cyclase (49,94). In the CPu, these two DA receptor subtypes appear to reside on the same cell and reciprocally regulate cyclic AMP generation (47,94). This suggests that the relationship between the different DA receptor subtypes has functional significance (76,103).

The importance of studying the interactive relationship between the different DA receptors is evident when the fact that endogenous DA acts at both receptor subtypes is considered (48). AMPH acts mainly by releasing presynaptic DA (65), and therefore also has indirect actions at both DA receptor subtypes (48). In addition, apomorphine has affinity for both D_1 and D_2 DA receptors (94) and it has been suggested that stimulation of both types of DA receptors may be necessary for the full expression of the effects of these DA agonists (10).

Thus, it would seem important to assess the dynamics of both receptor subtypes and their reciprocal relationship, at least in the CPu, when attempting to elucidate the underlying substrate for changes in DA-dependent behaviour. In Experiment 4, the effects of both D_1 and D_2 receptor stimulation on the activity of adenylate cyclase in the CPu was measured in isolation and socially reared rats. The first part of this experiment measured the accumulation of [^{3}H]cyclic AMP in response to DA stimulation of CPu slices in the presence of the D_2 receptor antagonist sulpiride. The second part measured the stimulation of [^{3}H]cyclic AMP accumulation by the specific D_1 receptor agonist 2,3,4,5-tetrahydro-2, 8-dihydroxy-1-phenyl-1 *H*-3-benzazepin (SK&F 38393) and the inhibition of this stimulation by the specific D_2 agonist quinperole hydrochloride (LY 171555). Unfortunately, it was not possible to assess DA receptor function in the NAC due to the small amount of NAC tissue available.

METHOD

Subjects

Subjects were of 32 female Lister hooded rats. Sixteen were socially reared and 16 reared in isolation for 12 weeks, as described in Experiment 1, except underhanging sawdust trays were not used so subjects had to be removed from their cages for cleaning.

Measurement of [^{3}H]Cyclic AMP Accumulation Following DA Stimulation in the Presence of Sulpiride

Cyclic AMP production was determined using a [^{3}H]adenine prelabelling technique. Rats were killed by cervical dislocation and decapitation. The whole CPu was dissected and slices (350 × 350 μm) were prepared using a McIlwain chopper (Mickle Laboratory Engineering Co., Surrey, UK). For each of four separate experiments, two socially reared and two isolation-reared rats were assayed with the CPu from each pair of rats combined. Slices were placed in a small volumetric flask and washed with Krebs–Henseleit solution (pH 7.4) containing 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 25.0 mM $NaHCO_3$, and 5.5 mM glucose and continually gassed with O_2/CO_2 (95:5%). The tissue slices were then incubated in 10 ml Krebs solution containing 1 mM 3-isobutyl-1-methylxanthine (IBMX) as a phosphodiesterase inhibitor in a shaking waterbath at 37° C for 1 h. The Krebs medium was changed every 20 min. The Krebs solution was then decanted and the tissue slices resuspended in 10 ml Krebs medium (+IBMX) containing 40 μl [8-^{3}H]adenine (26 Ci/mM) and the incubation continued for another 40 min. The prelabelled slices were then washed (3×) with 20 ml Krebs solution containing IBMX to remove excess [8-^{3}H]adenine. The slices were then allowed to settle.

Twenty-five-microlitre portions of the CPu slices were added to a test tube containing 255 μl Krebs medium and 10

μl sulpiride (1.5 mM, to give a final concentration of 50 μM). Each tube was gassed with O_2/CO_2 and incubated for 20 min at 37° C to allow equilibration. DA was then added (10 μl) and each tube regassed and incubated for a further 20 min. The final DA concentration in the incubation mix was either 0, 2.7, 9, 30, or 100 μM.

The incubations were stopped by adding 200 μl 1 M HCl and the tubes placed on ice for 20 min before adding 750 μl ice-cold distilled water. Tubes were then centrifuged for 15 min at 400 rev/min and a 1-ml sample of the aqueous supernatant was taken for the analysis of [^{3}H]cyclic AMP by column chromatography.

Sequential Dowex-alumina chromatography was used to isolate the [^{3}H]cyclic AMP. One millilitre of each sample was added to a test-tube and 100 μl [^{14}C]cyclic AMP was added to be able to correct for different recoveries from each column. Samples were applied to Dowex 50 ion exchange resin (0.6 ml; Sigma) in plastic Econo columns (Bio-Rad, Laboratories, Hercules, CA) that had been previously treated with 3 ml 1 M HCl and 10 ml distilled water. The columns were washed with a further 2 ml distilled water and the eluate discarded. The columns were then placed directly above similar plastic columns containing 0.6 g neutral alumina (Sigma), which had been previously washed with 10 ml 0.1 M imidazole. A further 4 ml distilled water was added to the Dowex columns, which eluted the [^{3}H]cyclic AMP onto the alumina columns. These alumina columns were then placed above scintillant vials and the [^{3}H]cyclic AMP was eluted in 5 ml 0.1 M imidazole. Ten millilitres of emulsifying scintillant (Optiphase X; LKB Ltd.) was added to each vial, which were then shaken vigorously and radioactivity determined by liquid scintillation counting.

Effect of Stimulation by SK&F 38393 and LY 171555 on the Accumulation of [^{3}H]cyclic AMP

The relationship between D_1 and D_2 dopamine receptors regulation of CPu adenylate cyclase activity was investigated. The specific D_1 receptor agonist SK&F 38393 was added alone and in conjunction with the D_2 receptor agonist LY 171555.

The method was as described above except the CPu from one rat from each of the rearing condition groups was used in each experiment and five separate experiments were carried out. The concentrations of DA agonists used in this experiment were based upon previous studies (47). The concentration of SK&F 38393 was 1 μM and that of LY 171555 10 μM. Sulpiride was not added to the incubation mix in these experiments.

Drugs

SK&F 38393 was obtained from Semat Ltd., St. Albans, UK. LY 171555 was a gift from Lily Research Laboratories. [^{3}H]- and [^{14}C]cyclic AMP were obtained from Amersham International (Amersham, UK).

RESULTS

Isolated rats were spontaneously hyperactive compared to socially reared controls, $F(1, 30) = 14.27$, $p < 0.001$, confirming that the slightly different rearing conditions produced equivalent effects on spontaneous activity to those described in the previous experiments.

Effects of Social Isolation on DA-Stimulated Cyclic AMP Production in the Presence of Sulpiride

Stimulation of CPu D_1 receptors by DA (0, 2.7, 9.0, and 30 μM) in the presence of the specific dopamine D_2 receptor antagonist (-)-sulpiride (50 μM) produced a marked enhancement in cyclic AMP accumulation (Fig. 6). This increase in cyclic AMP accumulation reached a maximum of approximately 240% of the basal level, $F(3, 12) = 24.73$, $p < 0.001$. Rearing rats in isolation did not affect either the baseline level of cyclic AMP production, $F(1, 20) = 0.72$, n.s. (mean dpm: social, 2,673; isolates, 2,732), or the stimulation of adenylate cyclase by activation of D_1 receptors by DA, $F(3, 12) = 1.54$, n.s., when compared to socially reared rats.

Effects of Social Isolation on D_2 Receptor Inhibition of D_1-Stimulated Cyclic AMP Accumulation

In confirmation of the results in the first part of this experiment, social isolation did not significantly alter basal levels of cyclic AMP accumulation in CPu slices, $t(8) = 0.43$, n.s. The selective D_1 receptor agonist SK&F 38393 increased cyclic AMP accumulation, which was reduced by addition of the selective D_2 agonist LY 171555, $F(1, 8) = 104.32$, $p < 0.001$ (Fig. 6). Isolation-reared rats did not differ from socially

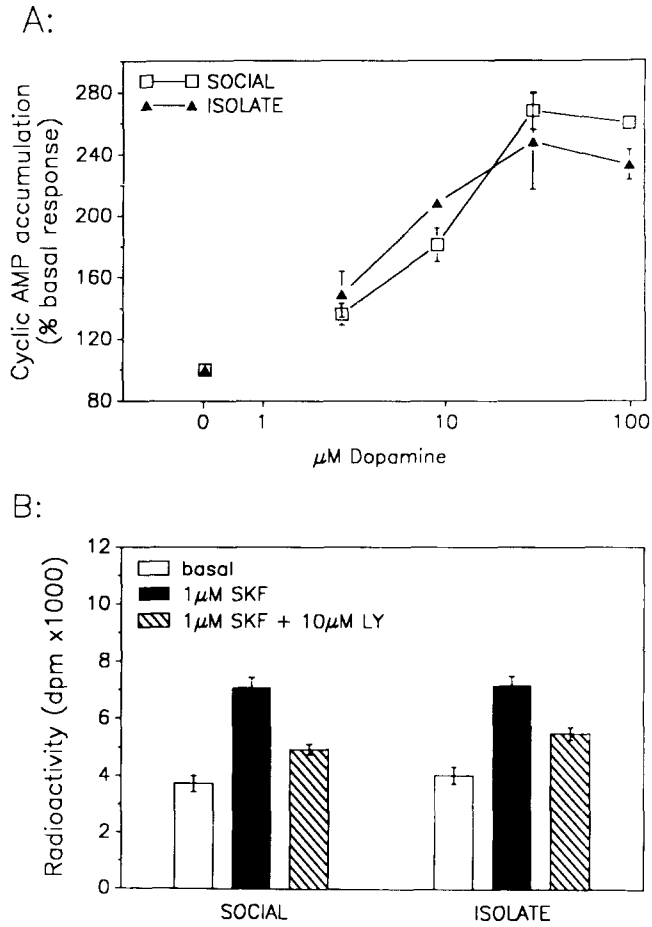

FIG. 6. (A) Effect of isolation rearing on DA-stimulated cyclic AMP accumulation in the presence of 50 μM (-)-sulpiride. Values are the mean ± SEM for four separate experiments with two rats from each rearing condition group in each experiment. (B) Effect of isolation rearing on D_1 and D_2 receptor stimulation of cyclic AMP accumulation. Slices were incubated with either no drug (basal) or in the presence of 1 μM SK&F 38393, or both 1 μM SK&F 38393 and 10 μM LY 171555. Values are the mean ± SEM from five separate experiments.

reared controls either in the presence of SK&F 38393 alone or in combination with LY 171555, $F(1, 8) = 1.19$, n.s. The percentage decrease in cyclic AMP accumulation due to LY 171555 was also calculated from the level in the presence of SK&F 38393 alone. Social isolation also did not affect this measure of the functional coupling between the two receptor subtypes, $F(1, 8) = 1.97$, n.s.

DISCUSSION

These results suggest that the effects of isolation on dopaminergic function in the CPu do not appear to involve postsynaptic mechanisms. There were no significant effects of rearing condition on either D_1 receptor stimulation of adenylate cyclase activity or on the attenuation of the D_1-stimulated activity by activation of D_2 receptors. Thus, the functional coupling between the two DA receptor subtypes (47,94) was apparently not influenced by rearing condition.

The results are in apparent contrast to previous reports of increased [^{3}H]spiroperidol binding in the striata from isolated rats (33). However, this study (33) used long-term isolation conditions (12–15 months) whereas the current experiment used 3 months of isolation. Age is known to influence dopaminergic mechanisms (79,100) and their modulation by social isolation (13,70). There is also thought to be an interaction between age and the effects of exercise on CPu DA function (61), and paucity of movement is implicated in at least some of the effects of isolation (97). In confirmation of the present results, isolation in mice does not alter striatal DA D_2 receptor binding (106). Increased striatal neuronal sensitivity in response to ionotophoretically applied DA has been reported following 3, but not 12, months of isolation (70).

In Experiment 1, apomorphine-induced stereotypy was reduced, particularly when the behaviour was maximally intense. Although the present results have not revealed a significant reduction in CPu postsynaptic mechanisms in isolates, there is a trend for the maximal stimulation of D_1 receptors to produce a reduced accumulation of cAMP in isolation-reared rats.

GENERAL DISCUSSION

The results from the present experiments provide clear evidence for altered monoamine function following isolation rearing in rats both indirectly in terms of the behavioural response to DAergic drugs and directly via neurochemical measures including in vivo dialysis. The most consistent finding is the isolation-induced reduction in 5-HT activity in the NAC. The postmortem 5-HIAA/5-HT ratio was reduced in Experiment 2, as was the basal extracellular level of 5-HIAA in Experiment 3, at both 3 and 9 months. This apparent reduction in 5-HT activity was only present in the NAC, consistent with earlier findings from this laboratory (41).

The reduction in 5-HT activity in the NAC may, at least in part, underlie the locomotor hyperactivity reliably demonstrated following isolation rearing of rats (44,66,82). 5-HT activity is thought to have an inhibitory effect on locomotor activity and motor responses in general (32,99). Intraventricular administration of 5-HT reduces (32), and the serotonin-depleting neurotoxin, 5,7-dihyroxytryptamine (5,7-DHT) increases (58), spontaneous locomotor activity. 5-HT within the NAC is thought to have a tonic inhibitory influence on DA function in this structure (11). Although microinjections of 5-HT directly into the NAC do not affect spontaneous locomotor activity (75), they do reduce locomotor activity induced by intraaccumbens DA (43) or AMPH (14,58). Furthermore, 5,7-DHT lesions of the NAC also increase both spontaneous and AMPH-induced locomotor activity (11,58). Biochemical evidence also suggests a modulatory role for 5-HT on DA function in the NAC. It was recently demonstrated that K^+-stimulated release of DA in synaptosomes from the NAC is regulated not only by presynaptic autoreceptors but by 5-HT receptors (37). Raphe lesions, which reduce forebrain 5-HT concentrations, increase the concentration of DOPAC in the NAC (36). The reduction in 5-HT activity in the NAC in isolated rats, together with the increased responsiveness of DA, suggests a mechanism for the alteration in locomotor response to systemic or intraaccumbens AMPH (40). This view is encouraged by reports that increasing 5-HT activity can reduce isolation-induced hyperactivity (27,71).

It is also interesting to note that depletion of cerebral 5-HT results in hyperphagia and increased weight gain (84), which are also consistently reported following social isolation in rats (25,66,67,88). Disruption of NAC DA function can also lead to transient hyperphagic responses, probably due to an inability to switch from one behavior to another (51).

This reduction in NAC 5-HT activity may contribute to other isolation-induced disturbances, such as the difficulty in withholding responses. Rats depleted of 5-HT are impaired in passive avoidance learning and, like isolates (29), are more reactive in arousing situations (91). The similarity between the behavioural effects of 5-HT depletion and those of isolation rearing is striking. For example, 5-HT blockade "results in a shift of behaviour toward facilitation of responding" (91). This description is similar to the pattern of behavioural disturbances following isolation rearing (40,41,67). The present results have demonstrated a possible neural locus for these effects.

In the NAC, there was an enhanced release of DA in response to amphetamine in isolates although the effect was not as large as in the CPu. However, the decrease in 5-HT activity may amplify this effect, as 5-HT release may also inhibit the action of DA activity downstream from the DA nerve terminal itself (58). Unfortunately, it was not possible to assess postsynaptic DA function in the NAC directly in these experiments although evidence from Experiment 1 would suggest that there is little change.

These results also provide a possible mechanism for the alteration in behavioural stereotypy induced by psychomotor stimulant drugs (82). Isolated rats showed disruption in pre- but not postsynaptic function, at least in the CPu, as indicated by increased extracellular DA in response to AMPH but no change in D_1 or D_2 receptor stimulation of adenylate cyclase.

It is also evident that isolation-induced increases in amphetamine stereotypy (22,82) are more reproducible than changes in apomorphine-induced stereotypy (13), which suggests that presynaptic mechanisms are influenced more strongly by isolation. Indeed, the pattern of stereotyped behaviours produced by isolation is different to that observed following pharmacological treatments that upregulate postsynaptic mechanisms (17). The lack of effect on D_1 or D_2 receptors, as well as the effect of apomorphine, would suggest that isolation would not affect behavioural responses to specific D_1 or D_2 agonists or antagonists. Shorter latencies to engage in tail-pinch-induced behaviours, and a reduced susceptibility to the disrupting effects of α-flupenthixol (81), are also consistent with increased presynaptic DA function in the CPu.

The increase in PFC dopamine has confirmed previous reports of isolation-induced disruption in DA mechanisms in

this structure (5,70). As DA activity in the PFC is thought to be inhibitory on subcortical DA projections (12), reductions in this activity may contribute to the elevated amphetamine response in the NAC and CPu in isolates.

DA-depleting lesions of the PFC can also lead to cognitive impairments (6) and a direct relationship between DA and the PFC and learning ability has been demonstrated (83). It is therefore possible that changes in PFC DA contribute to learning impairments in socially isolated rats (41,66).

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