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Isolation Rearing in Rats: Pre- and Postsynaptic Changes in Striatal Dopaminergic Systems

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HALL, F. S., L. S. WILKINSON, T. HUMBY, W. INGLIS, D. A. KENDALL, C. A. MARSDEN AND T. W. ROBBINS. *Isolation rearing in rats: Pre- and postsynaptic changes in striatal dopaminergic systems*. PHARMACOL BIOCHEM BEHAV **59**(4) 859–872, 1998.—Isolation rearing of rats produces a behavioral syndrome indicative of altered dopamine (DA) function in the nucleus accumbens (NAC). The present experiments extend these findings by investigating: (a) interactions between isolation rearing and repeated handling/testing on presynaptic DA function in the NAC using in vivo microdialysis; (b) the dose–response curve for the effects of *d*-amphetamine, and the responses elicited by high potassium, using in vivo microdialysis; and (c) postsynaptic function in isolates as indexed by DA receptor-linked cAMP production. Experiment 1 showed that both isolation rearing and repeated handling/testing had effects on monoamine function in the NAC. However, while both manipulations enhanced DA release evoked by *d*-amphetamine, only isolated rats had elevated basal DA levels. Opposite neurochemical changes were observed with respect to the serotonin metabolite 5-HIAA, isolates having lower, and repeatedly handled/tested animals having higher, extracellular levels. Experiment 2 provided evidence for enhanced *d*-amphetamine–evoked DA release in isolated animals, while potassium-evoked DA release was reduced. Experiment 3 provided evidence that the isolation rearing induced changes in presynaptic DA function were accompanied by postsynaptic changes. Specifically, the inhibitory influence of the D_2 receptor on D_1 receptor-stimulated cAMP production was attenuated in ventral striatal slices taken from isolates, suggesting a functional downregulation of D_2 receptors. © 1998 Elsevier Science Inc.

Isolation rearing Dopamine Nucleus accumbens In vivo microdialysis cAMP

REARING rats in social isolation produces a syndrome of effects that includes hyperactivity (6,10,36), increased exploration (29), heightened response to dopamine agonists (5,17,19,30), heightened response to conditioned and unconditioned reinforcers (19,32), impaired acquisition of schedule-induced-polydipsia (20), decreased pain thresholds (28), and perseveration (7,18,24). Many of these behavioral effects implicate alterations in dopamine (DA) function, and in particular, DA function in the nucleus accumbens (NAC). Measurements of whole tissue neurotransmitter levels in selected brain regions have provided inconsistent results (1,17,18,37,38). Recently, however, using in vivo microdialysis methods that allow measurements of extracellular levels of neuroactive substances, it has been established that socially isolated rats show enhanced levels of extracellular dopamine in the nucleus accumbens following systemic *d*-amphetamine (17,40).

Despite the overall consistency of isolation-induced effects on behavior thought to involve brain dopamine systems, there are some apparently contradictory findings. For example, while Phillips et al. (26,27) found a decreased propensity to self-administer cocaine in isolates, previous work by Schenk et al. (33) found no difference with respect to the self-administration of *d*-amphetamine. In addition, the place preference behavior produced by *d*-amphetamine was also unaffected by social isolation (31). There are a number of possible reasons for these discrepancies, including (a) methodological differences with respect to additional procedures carried out during the isolation period; (b) the possibility that different behavioral circumstances impact selectively on underlying isolation-induced changes in presynaptic ventral striatal dopamine function, not always giving rise to enhanced function; and (c) complex interactions between a specific behavioral context, underlying changes in presynaptic function and possible adaptive changes in postsynaptic dopamine function. In Experi-

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ment 1 of the present work we, therefore, examined the effects of repeated handling/behavioral testing on neurochemical and behavioral effects of isolation rearing. In Experiment 2, the dose dependency of the effects of systemic *d*-amphetamine on extracellular dopamine release in isolates was examined and a comparison made with the effects of local depolarizing levels of potassium. Experiment 3 was designed to test whether any presynaptic changes in striatal dopamine function were accompanied by changes in postsynaptic dopamine function.

EXPERIMENT 1

As noted above, some of the discrepancies in the isolation literature may be due to the confounding effects of other experiences, for instance, repeated handling/testing such as is necessitated by some experimental designs. Repeated handling/testing has been reported to change the behavior of socially reared rats (16,39) and isolation-reared rats (7). The present experiment examined the impact of repeated handling/testing on a highly reproducible behavioral effect of isolation, locomotor hyperactivity (6,10,20,36), and also extracellular DA following *d*-amphetamine administration as measured with in vivo microdialysis. In these previous studies (17,40) the subjects had not experienced extensive behavioral testing. Because many paradigms of interest involve extensive handling, the possible interaction between the procedures involved in repeated behavioral testing and isolation rearing were investigated. The anesthetized paradigm was used so that differences in locomotor activity between groups would not influence neurotransmitter levels and confound the results, and Halothane does not substantially affect dopamine function (9).

METHOD

Subjects

Subjects were 24 Lister hooded male rats, received from the supplier (Harlan Olac, Bicester, UK) on day 21 postnatal and randomly divided into rearing, isolation-reared (ISO) and socially reared (SOC), and repeated testing/handling conditions, not handled (NH), or repeated handling (RH). Half of the subjects were reared socially, 4 per 56 × 38 × 18 cm cage, and half in isolation, 1 per 45 × 20 × 20 cm cage. The plastic cages had steel grid floors. All subjects were housed in the same room with a 12 L:12 D cycle and ad lib food and water. All subjects could see, hear, and smell other rats so that the isolation conditions only prevented physical contact. Half of the subjects in each rearing condition were tested three times per week for locomotor activity between days 4 and 84 postweaning. Unhandled subjects were tested for locomotor activity once, on day 85 postweaning.

Locomotor Activity

Locomotor activity was assessed in activity chambers between 1400 and 1600 h. Isolation-reared rats have previously been shown to be hyperactive in this apparatus. These boxes (45 × 25 × 18 cm) had two infrared photobeams positioned across the box, 6 cm from each end, 1 cm above the floor. The boxes were controlled, and data collected, via a BBC microcomputer programmed in BBC Basic. Both total beam breaks and consecutive beam breaks (runs) were collected. "Runs" required the subject to break the infrared beam at each end of the box in sequence, providing an estimate of locomotion as opposed to general activity. Subjects were tested for 2 h and the data divided into 12 10-min time bins.

In Vivo Microdialysis of the NAC

In vivo microdialysis was performed under anesthetized conditions after 12 weeks. The subjects were placed in a stereotaxic frame modified to contain an airtight box that fitted over the incisor bar of the stereotaxic instrument. A Halothane/oxygen mixture (0.9% Halothane/0.4 liters oxygen per minute) was passed into the box and out again via negative pressure. Core body temperature was monitored using a rectal thermometer and was maintained at 35°C using a heating pad. Microdialysis probes, constructed in house, were of concentric design with a 2-mm dialyzing region. The dialysis membrane was Hospal (molecular weight cutoff 50,000 Daltons, external diameter 250 μm). In vitro studies found that recovery of the probe for DA was approximately 15% at a 2 μl per minute flow rate. Dialysis probes were unilaterally implanted in the NAC. The coordinates were AP +3.4, ML ±1.5, DV −7.8 (Pellegrino and Cushman, 1967) with the incisor bar set at +5.0 mm. Artificial CSF (4.0 mM KCl, 147.0 mM NaCl, 1.4 mM $CaCl_2$, 1.0 mM $MgCl_2$, pH 6.5) was perfused at 2.0 μl/min. Microdialysis samples were collected at 30-min intervals beginning 3 h after probe implantation. After three baseline samples were collected the subjects were given 2.0 mg/kg *d*-amphetamine SC (Sigma, St. Louis, MO) and sampling continued for 4 more hours. At the end of the experiment the subjects were overdosed with pentobarbital, the brains removed, and postfixed in 4% paraformaldehyde. The brains were sectioned horizontally and stained with cresyl violet to localize the microdialysis probe placements.

HPLC Analysis

All samples were analyzed by HPLC-EC for DA, 5-hydroxyindoleacetic acid (5-HIAA), and 3,4-dihydroxyphenylacetic acid (DOPAC) levels using a 1 mm column (Spherisorb, ODS5). The mobile phase consisted of 152 mM citric acid, 15 mM sodium acetate, 1.98 mM octane sulphonic acid, 0.8 mM EDTA, 8% methanol, pH 3.6. Following chromatographic separation the compounds of interest were quantified by amperometric electrochemical detection (+0.7 V) using a TRIO chromatographic work station.

Data Analysis

Weight data from the final measurement were subjected to analysis of variance (ANOVA) with two between-subjects factors—rearing and handling. Locomotor activity data for sessions 1, 7, and 13 were analyzed separately by ANOVA with one between-subjects factor, rearing, and time as a repeated factor. Total runs and breaks for the unhandled subjects were analyzed by a *t*-test. For each compound of interest, the dialysis data were analyzed using a three-way ANOVA with two between-subjects factors, rearing and handling, and one repeated factor, time. Separate analyses were performed on the basal data, the amphetamine data, and the data as a whole. Although differences in basal dopamine levels were observed, analysis of the amphetamine effect by converting the scores to percent differences found the same pattern of effects produced by rearing and handling, so for brevity only, the statistical analysis of the actual values is presented.

RESULTS

Body Weight

After 12 weeks of rearing, the mean weight of ISO NH rats was 345.4 ± 9.1 g, while the mean weight of SOC NH rats was

326.7 ± 3.6 g. In contrast, the mean weight of ISO RH rats was 334.5 ± 7.5 g, while the mean weight of SOC RH rats was 328.2 ± 7.8 g. Thus, while the ISO NH rats were heavier than their controls, repeated handling/testing appeared to eliminate this difference in weight. However, neither of the main effects, rearing, $F(1, 16) = 2.69$, NS, or handling, $F(1, 16) < 1.0$, NS, nor the interaction between rearing and handling, $F(1, 16) < 1.0$, NS, were significant.

Locomotor Activity

The locomotor activity scores of the RH subjects for sessions 1, 7, and 13 are represented in Fig. 1. After 4 days of isolation (session 1) ISO rats exhibited greater activity than SOC controls for breaks and runs, which was confirmed by a significant main effect of rearing for both measures [breaks, $p(1, 10) = 9.77$, $p < 0.02$, Fig. 1A; runs, $F(1, 10) = 17.33$, $p < 0.002$, Fig. 1D].

The hyperactivity exhibited by ISO rats persisted for some time. They were still more active than their SOC controls in the seventh test session, at day 24 postweaning. The ANOVA for locomotor activity demonstrated a significant main effect of rearing for breaks, $F(1, 10) = 8.05$, $p < 0.02$ (Fig. 1B), and runs, $F(1, 10) = 7.88$, $p < 0.02$ (Fig. 1E).

By test session 13, at day 44 post-isolation, there were no differences in activity between the rearing groups. The factor of rearing was not significant in the ANOVA for either beam breaks (Fig. 1C) or runs (Fig. 1F).

After 8 weeks of rearing, ISO NH rats exhibited a tendency to be hyperactive (breaks, 1812 ± 450; runs, 283 ± 95) compared to SOC NH controls (breaks, 1113 ± 301; runs, 145 ± 59), although these differences did not reach significance ($p < 0.10$). Unfortunately, a computer error prevented assessment of the time course of locomotor activity in these subjects.

Neurochemical Findings

All microdialysis probes were located in the NAC (Fig. 2). Placements are represented in the horizontal plane because there was very little variance in the dorsal–ventral placement coordinates. As can be seen, the probe placements were distributed between both the shell and core regions of the NAC.

ISO rats had higher basal DA levels than SOC controls,

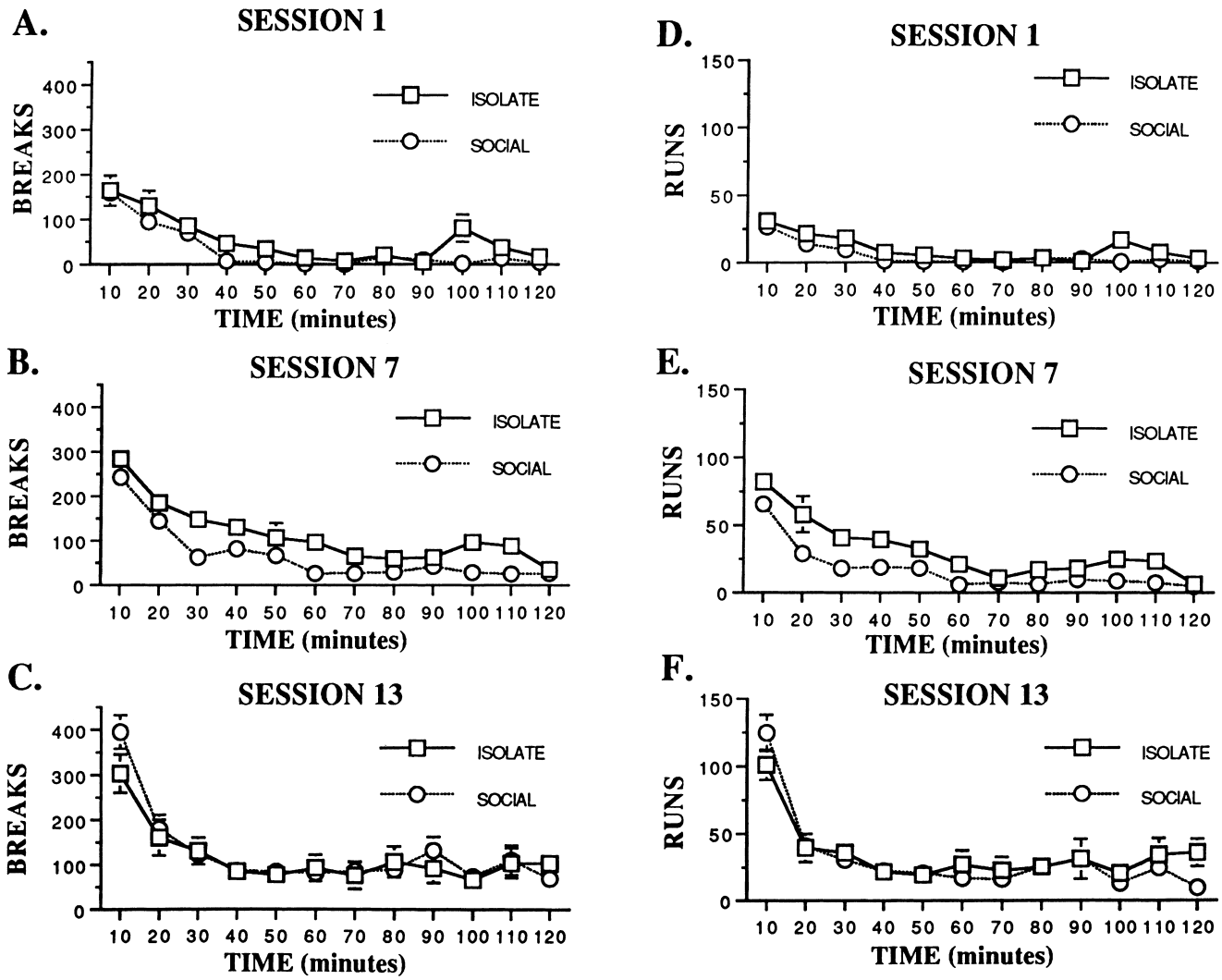

FIG. 1. The time course of activity for isolation-reared and socially reared rats for session 1 (A and D), session 7 (B and E) and session 13 (C and F) for breaks and runs respectively. Data are expressed as mean ± standard error.

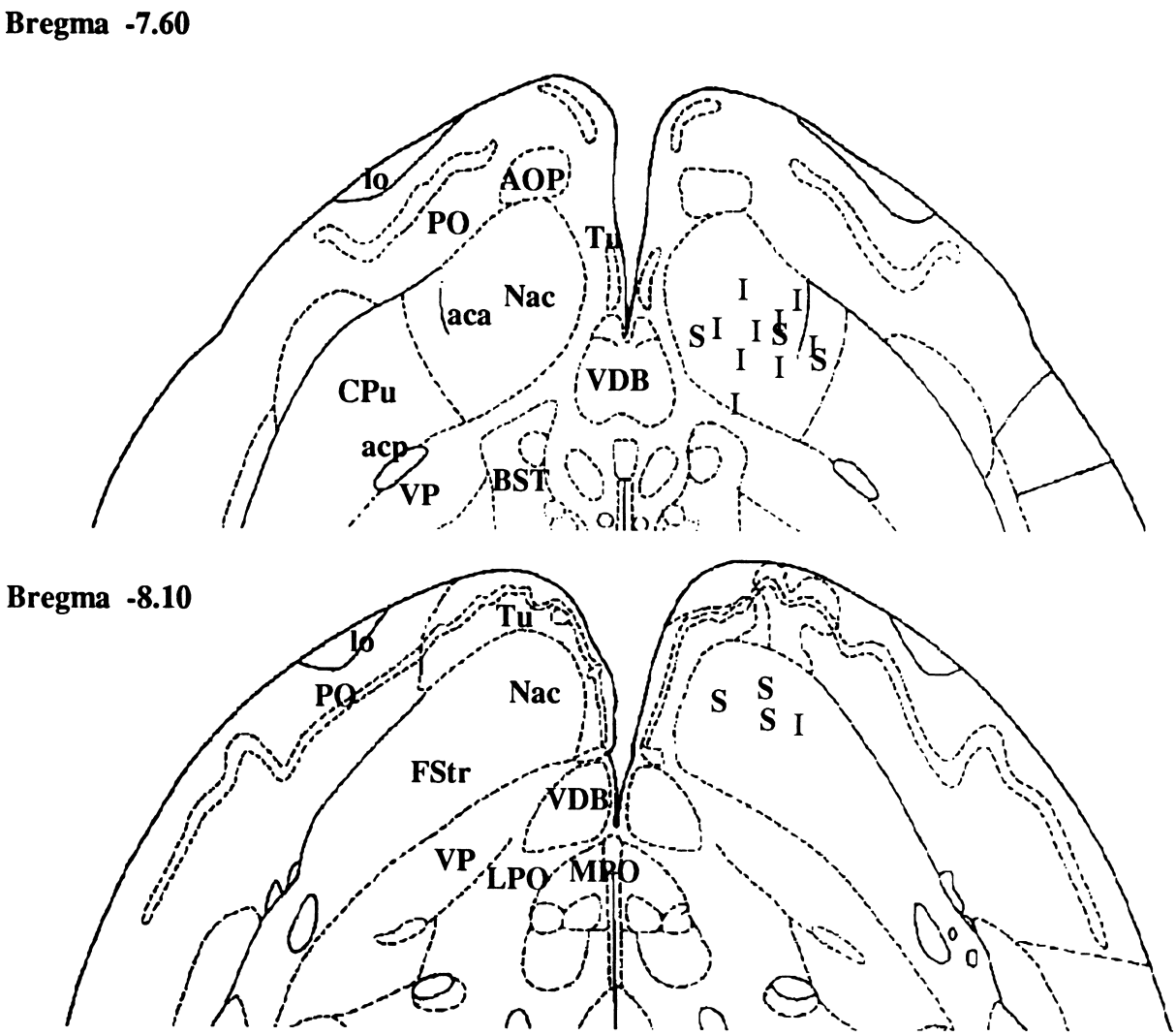

FIG. 2. Histologically verified placement of microdialysis probes in the brain represented on horizontal sections from the atlas of Paxinos and Watson (1986), −8.10 and −7.60 mm from bregma: S—socially reared. I—isolation-reared. Anatomical areas: aca—anterior commisure, anterior, acp—anterior commisure, posterior, AOP—anterior olfactory cortex, BST—bed nucleus of the stria terminalis, CPu—caudate-putamen, FStr—fundus striati, lo—optic tract, LPO—lateral preoptic area, MPO—medial preoptic area, Nac—nucleus accumbens, Tu—olfactory tubercle, VDB—ventral diagonal band, VP—ventral pallidum.

$F(1, 17) = 28.46, p < 0.0001$ (Fig. 3A inset), but repeated handling/testing was without effect. However, analysis of the postamphetamine data indicated that both rearing, $F(1, 17) = 11.83$, $p < 0.004$, and handling, $F(1, 17) = 13.93, p < 0.002$, increased *d*-amphetamine evoked DA release in the NAC (Fig. 3A). However, the interaction between rearing and handling was not significant, indicating that rearing and handling had independent but additive effects on NAC DA release, $F(1, 14) < 1.0$, NS.

RH rats had higher basal DOPAC levels than NH rats, $F(1, 17) = 4.87, p < 0.04$ (Fig. 3B). After *d*-amphetamine, RH rats still had higher DOPAC levels, $F(1, 17) = 6.70, p < 0.02$, although there was a steep decrease in extracellular DOPAC postamphetamine in all groups with no indication of any differential responses to the drug. By contrast, there was no significant effect of rearing on basal DOPAC levels, or postamphetamine DOPAC levels. However, the increased basal DOPAC levels found in RH rats were even greater in rats that had also experienced isolation rearing, as demonstrated by a significant interaction between handling and rearing, $F(4, 56) = 3.98, p < 0.007$.

Basal 5-HIAA levels were greater in RH rats, $F(1, 17) = 4.46, p < 0.05$ (Fig. 3C), but were not significantly affected by isolation rearing. Nonetheless, it is interesting to note that ISO RH 5-HIAA levels were higher that SOC RH levels, while ISO NH levels were lower than SOC RH levels, which may be a tentative indication of an interaction. *d*-Amphetamine administration also did not affect 5-HIAA levels.

DISCUSSION

The present experiment has replicated previous findings in Lister hooded rats that isolation rearing produces hyperactivity in a novel environment (6) and increased weight gain (25). Furthermore, these data indicate that isolation rearing of rats increased basal levels of extracellular DA and confirm that

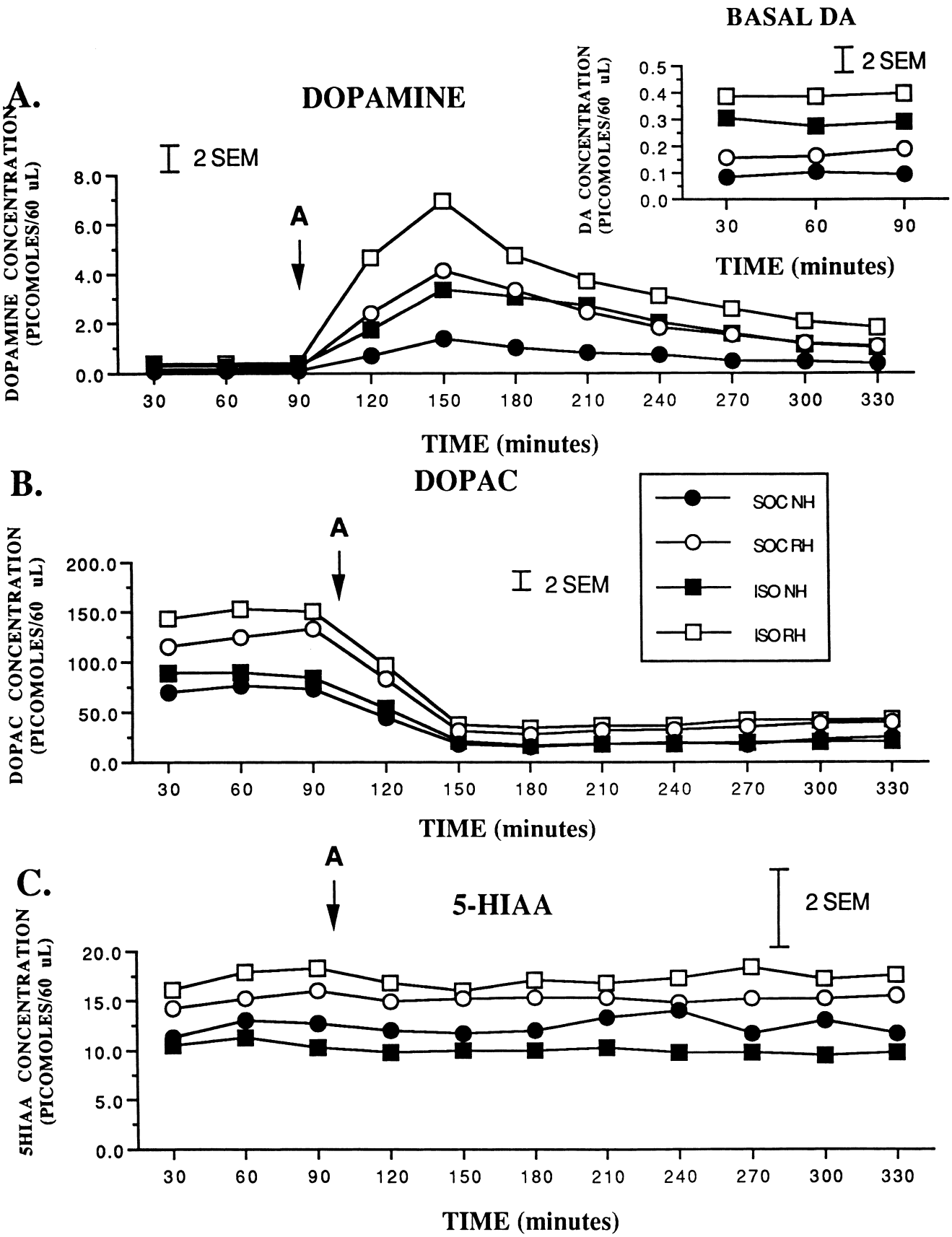

FIG. 3. Levels of DA (A), DOPAC (B), and 5-HIAA (C) in nucleus accumbens dialysates for all four groups; ISO—isolation reared, SOC—socially reared, RH—repeated handling, NH—nonhandled. *d*-Amphetamine (2.0 mg/kg SC) was administered after the point marked by the arrow. The error bars represent twice the standard error of the mean for the three-way interactions between rearing condition, handling/testing, and time. For dopamine, the SEM for both basal and *d*-amphetamine stimulated levels are included. The inset represents basal levels of DA (pmol/60 μl).

isolation rearing potentiates the effects of systemic *d*-amphetamine on DA release in the NAC (17,40).

In addition, this study has revealed that the repeated handling/testing of rats from the weaning age can also have profound and enduring effects on the dynamics of DA release in the NAC. In the case of DA release in the NAC, the effects of repeated handling/testing and isolation rearing were similar and additive. Thus, isolated handled rats exhibited the highest levels of extracellular NAC DA. Importantly, however, this was not the case with respect to other measures. For example, the weight gain of isolation-reared rats was reversed in subjects that experienced repeated handling/testing. A similar dissociation between the effects of the two kinds of early experience with respect to extracellular 5-HIAA levels in the NAC did not reach statistical significance. Hence, the overall conclusion is that while both isolation rearing and repeated handling/testing early in development can lead to similar behavioral and neurochemical changes, there are also important differences between them.

EXPERIMENT 2

From the results of the previous experiment and previous studies (17,40) it would seem established that isolation rearing potentiates *d*-amphetamine–induced release of DA in the NAC. However, all of these studies have used the single, same dose of *d*-amphetamine (i.e., 2 mg/kg). The possibility exists, therefore, that the effects of isolation rearing were peculiar to the particular dose of *d*-amphetamine used, rather than being due to a general change in the sensitivity of presynaptic DA function. To investigate this possibility the effects of a range of *d*-amphetamine doses on extracellular DA were assessed by in vivo microdialysis.

Additionally, to characterize further the generality of the effects of isolation rearing on presynaptic DA function in the NAC, the effects of a locally applied depolarizing pulse of potassium ions was examined in some subjects. The rationale was that neurotransmitter release evoked by high K^+ occurs by a mechanism that is fundamentally different from that of *d*-amphetamine (4). Finally, we sought to extend the generality of the previous results (17) (Experiment 1) by using Sprague–Dawley rather that Lister hooded rats for these experiments.

METHOD

Subjects

The subjects were 40 male Sprague–Dawley rats (Harlan Olac, Bicester, UK), randomly divided into rearing conditions at weaning (ISO or SOC). Subjects were housed as in Experiment 1 for 8 weeks before the experiment began.

Behavioral and Neurochemical Procedures

After 8 weeks of rearing the subjects were tested for locomotor activity as described previously. Subsequently, between 8 and 12 weeks postweaning, anesthetized microdialysis was performed as described previously except that each subject was injected with either saline or *d*-amphetamine sulfate (Sigma, St. Louis, MO) in doses of 0.5, 1.0, 2.0, or 4.0 mg/kg SC ($n = 4$ per group) counterbalanced across microdialysis sessions.

Prior to the injection of d-amphetamine some subjects ($n = 5$ ISO rats and $n = 5$ SOC rats) received two 5-min pulses of 75 mM K^+ perfusate administered via a liquid switch (CMA110, Carnegie Medicin). The sampling period was 30 min except during K^+ administration, when it was reduced to 10 min. After each potassium pulse six samples were collected, and following the second pulse at least 2 h elapsed before d-amphetamine administration. Dialysate samples were analyzed for dopamine, DOPAC, and 5-HIAA as described previously.

Data Analysis

Locomotor activity data were subjected to ANOVA with one between-subjects factor, rearing, and one repeated measure, time. *d*-Amphetamine data were expressed as the percentage difference from baseline for the maximal response. Analysis of the raw data by ANOVA failed to reveal any interaction between rearing and time. The *d*-amphetamine data were subjected to ANOVA with two between-subjects factors, rearing and *d*-amphetamine dose.

The absolute amounts of extracellular DA were used to assess the effects of isolation rearing on the response to K^+. These data were submitted to ANOVA with one between-subjects factor, rearing, and one repeated factor, time. Also, the maximal response from each potassium pulse was expressed in percentage terms as the difference from the three preceding baseline samples. This data was subjected to ANOVA with one between-subjects factor, rearing, and the repeated factor, potassium pulse.

RESULTS

Activity Results

Consistent with previous results, ISO rats were more active than SOC rats both in terms of breaks and runs (data not presented). This was confirmed by a significant main effect of rearing for breaks, $F(1, 38) = 6.45$, $p < 0.02$, and for runs, $F(1, 38) = 8.66$, $p < 0.005$.

Neurochemical Results

All microdialysis probe placements (Fig. 4) were located in the NAC and are represented in the horizontal plane because there was very little variance in the dorsal–ventral placement coordinates. Probe placements were distributed between both the shell and core regions of the NAC.

As expected, *d*-amphetamine produced substantial increases in extracellular DA in the NAC that were greater with increasing dose, $F(4, 32) = 15.15$, $p < 0.0001$ (Fig. 5A). At doses above 1 mg/kg the release of DA was potentiated in isolation-reared rats, $F(1, 32) = 4.47$, $p < 0.05$. However, as the dose–response curves for both groups of animals did not approach an asymptote it is difficult to conclude whether the between-group differences manifest at high doses of *d*-amphetamine were due to a left-ward shift in the dose–response curve or an increase in maximal drug-evoked release. Post hoc analysis revealed that the effect of rearing was only significant at the highest dose of *d*-amphetamine ($p < 0.05$, Newman–Keuls). There was no difference in the basal levels of DA in the NAC between-rearing groups (SOC 0.193 ± 0.043 pmol/60 μl, ISO 0.222 ± 0.056 pmol/60 μl).

As expected, *d*-amphetamine significantly decreased DOPAC levels in NAC dialysates, $F(4, 32) = 6.04$, $p < 0.002$ (Fig. 5B). However, rearing had no impact on *d*-amphetamine–induced decreases in DOPAC. Finally, there was no effect whatsoever of dose or rearing on 5-HIAA levels (Fig. 5C).

High K^+ in the dialysis perfusate increased the efflux of DA, as demonstrated by a significant effect of time in the ANOVA, $F(10, 80) = 10.25$, $p < 0.004$ (Fig. 6A). In apparent contrast to systemic *d*-amphetamine, ISO rats had a diminished response to 75 mM K^+ compared to SOC controls, $F(1, 8) = 7.35$, $p < 0.027$. Furthermore, the time course of DA efflux produced by high perfusate K^+ was delayed in ISO rats. In SOC subjects the peak response occurred in the first 10-

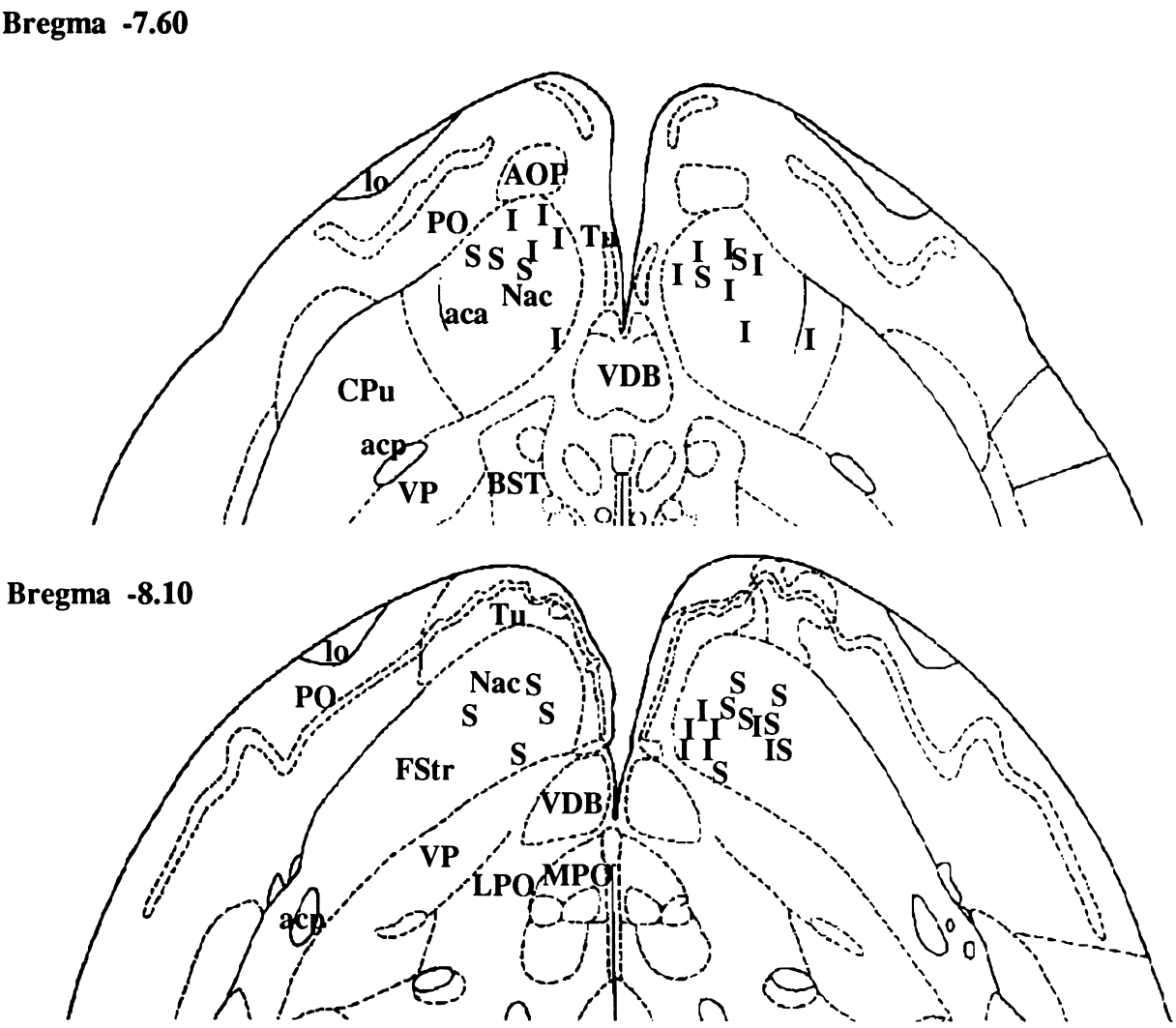

FIG. 4. Histologically verified placement of microdialysis probes in the brain represented on horizontal sections from the atlas of Paxinos and Watson (1986), −8.10 and −7.60 mm from the interaural line: S—socially reared subject, I—isolation-reared subject. Anatomical areas: aca—anterior commisure, anterior, acp—anterior commisure, posterior, AOP—anterior olfactory cortex; BST—bed nucleus of the stria terminalis, CPu—caudate-putamen. FStr—fundus striati, lo—optic tract, LPO—lateral preoptic area, MPO—medial preoptic area, Nac—nucleus accumbens, TU—olfactory tubercle, VDB—ventral diagonal band, VP—ventral palllidum.

min sample after K^+ injection, while in ISO subjects the peak response occurred in the second sample. Thus, there was a significant interaction between rearing and time, $F(8, 80) = 2.87$, $p < 0.004$. When the K^+ pulses were analyzed in percentage terms, although the effect of rearing was again significant, the two successive K^+ pulses produced equivalent release of DA.

The K^+ pulse also precipitated changes in the levels of monoamine metabolites in the sample immediately following the K^+ pulses (Fig. 6B and C). Analysis of the time course of metabolite levels during the K^+ pulses revealed significant effects of time for DOPAC, $F(10, 70) = 8.49$, $p < 0.004$, and 5-HIAA levels, $F(10, 70) = 9.12$, $p < 0.004$, but not of rearing.

DISCUSSION

The present experiment confirms that isolation rearing potentiates the effects of *d*-amphetamine on extracellular DA in the NAC as measured by in vivo microdialysis. However, the magnitude of the *d*-amphetamine response was somewhat less than in the previous experiment (compare Fig. 5A to Fig. 2A). The percentage increase at 2.0 mg/kg *d*-amphetamine in Experiment 1 was 1600% for socially reared rats compared to 2200% for isolation-reared rats. The same dose produced only a 615% increase in socially reared rats and a 1288% increase in isolates in Experiment 2. It is unlikely that methodological differences accounted for these differing results because the same equipment was used and they were executed contiguously. The most likely explanation for this difference is the strain used: Sprague–Dawley rats vs. Lister hooded rats. Furthermore, some of the changes induced by isolation rearing in Experiment 2 were attenuated compared to Experiment 1. For instance, lower basal DA levels were found in Experiment 2 for ISO rats (222 ± 0.056 pmol/60 μl), compared to the ISO NH rats in Experiment 1 (290 ± 0.115 pmol/60 μl). Also, the hyperactivity in Lister hooded rats (1812 ± 450 in

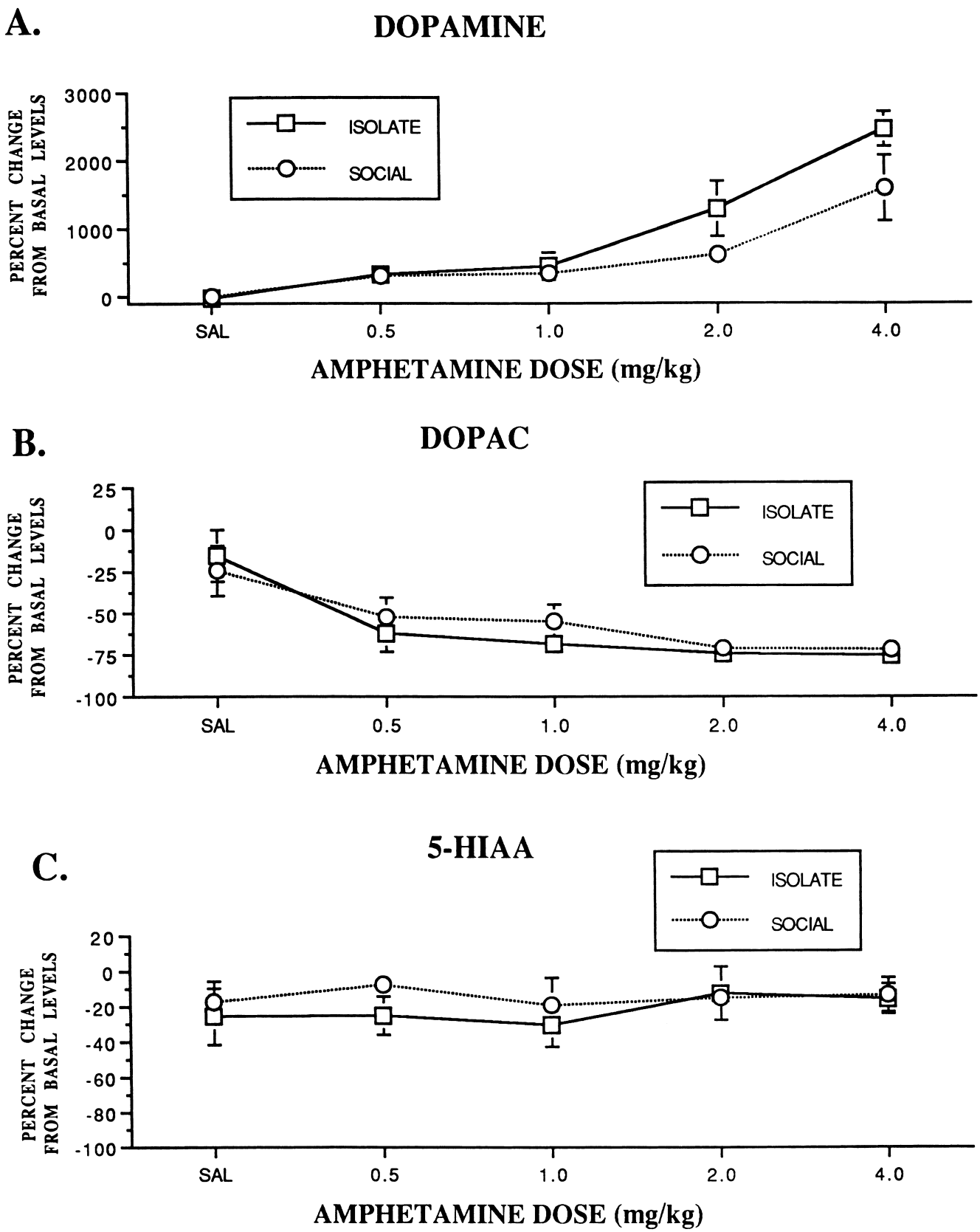

FIG. 5. The effect of *d*-amphetamine on the concentration of dopamine (A), DOPAC (B), and 5-HIAA (C) for isolation-reared rats (ISO) and socially reared rats (SOC), expressed as percentage change from basal levels following administration of four doses of *d*-amphetamine. Data are expressed as mean ± standard error.

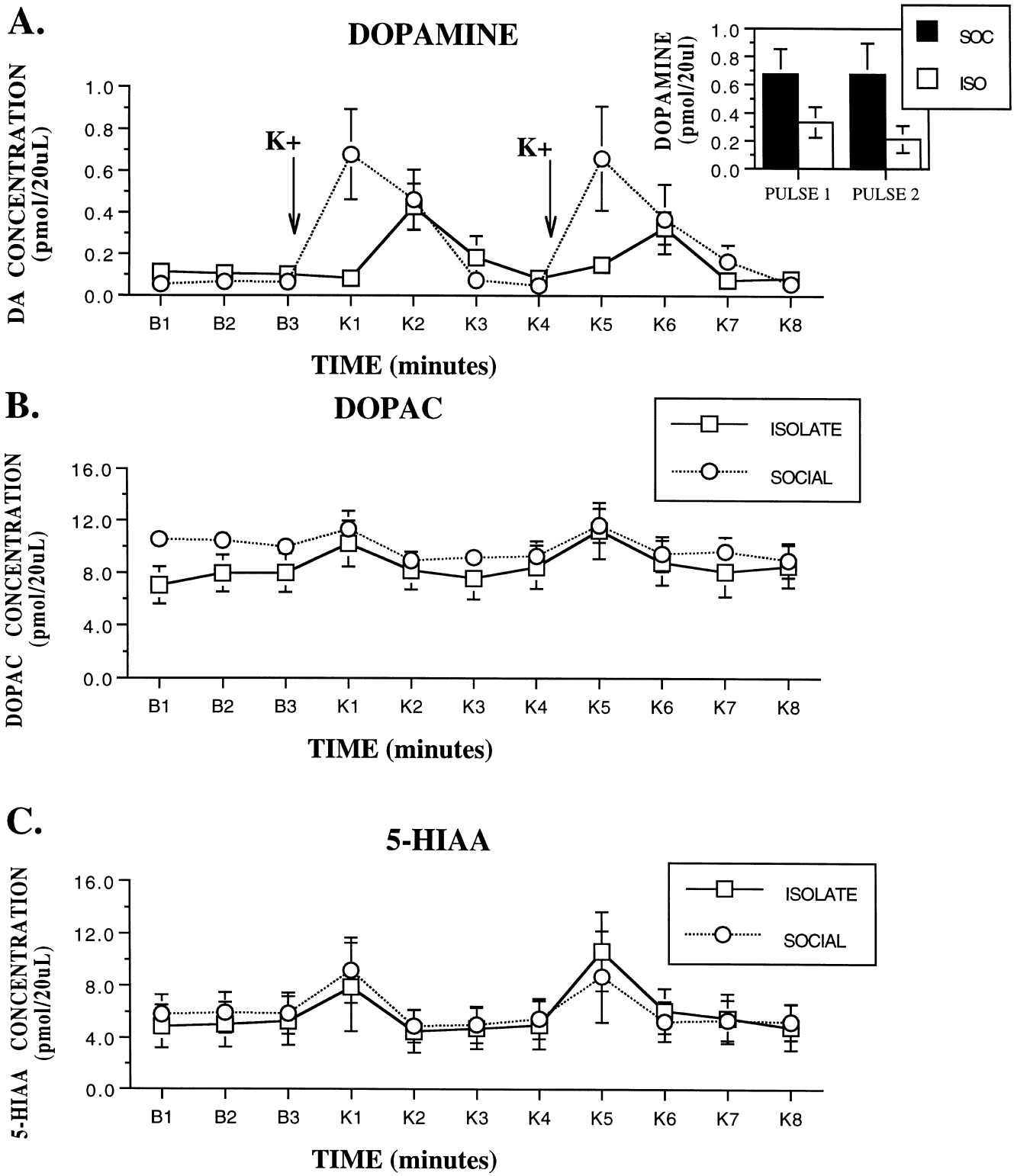

FIG. 6. Time course of the effect of increased perfusate K^+ on the concentration of dopamine (A), DOPAC (B), and 5-HIAA (C) in NAC perfusates for isolation-reared rats (ISO) and socially reared rats (SOC). Administration of local K^+ pulses are marked by the arrows. Data are represented as the mean ± standard error. Samples B1–B3 are baseline samples and samples K1–K8 are potassium samples. The inset represents the maximal percent increase in DA levels after the two potassium pulses ± standard error.

ISO rats vs. 1113 ± 301 SOC rats) was greater than that in the Sprague–Dawleys in Experiment 2 (1341 ± 55 in ISO rats vs. 1112 ± 68 in SOC rats).

Diminished sensitivity to increased perfusate K^+ in ISO rats has major implications for the nature of isolation-rearing changes in presynaptic DA function. In particular, the reduced impact of increased perfusate K^+ on DA efflux in ISO rats suggests that increased sensitivity to *d*-amphetamine was not simply the result of increased terminal density. If anything, the decreased impact of high K^+ perfusate might, a priori, indicate a decrease in the number of DA terminals. Regardless of the nature of this effect, isolation rearing does not appear to universally potentiate the action of agents which evoke DA release.

EXPERIMENT 3

A previous study reported that there were no differences between ISO and SOC rats in cAMP accumulation in dorsal striatal tissue slices in response to DA, SKF 38393, or LY17155. However, most evidence implicates changes in ventral striatal DA function in isolation-rearing effects. The following experiment examined D_1 receptor function in NAC and DS tissue slices, and in another group of rats, D_1 and D_2 receptor function in the NAC. In this study, D_1 receptor function was operationally defined as the amount of cAMP accumulation in the presence of the D_2 antagonist sulpiride, while D_2 receptor function was defined as the difference between this effect and the amount of cAMP accumulation in absence of sulpiride. The subjects were also tested for DA stereotypy and locomotor activity produced by apomorphine, to provide behavioral measures of DA receptor function.

METHOD

Subjects

The subjects were 24 SOC and 24 ISO male Lister hooded rats. All housing conditions were identical to those previously described, and the experiment began after 8 weeks of rearing.

Apomorphine-Induced Locomotor Activity and Stereotypy

Locomotor activity and stereotypy was assessed in a 1 × 1 m open field, which was divided into 25 equal squares. The number of squares entered was recorded. Subjects tested for stereotypy (n = 16 ISO, n = 16 SOC) were injected with apomorphine HCl (1.0 mg/kg SC, Sigma) or saline. A standard six-point stereotypy scale was used (3), and stereotypy was assessed every 5 min for 1 h, while locomotor activity was simultaneously assessed.

Measurement of [^{3}H]-cAMP Accumulation in Nucleus Accumbens and Dorsal Striatum Following DA Stimulation in the Presence of Sulpiride

After 12 weeks of isolation the subjects (n = 12 ISO, n = 12 SOC) were sacrificed by stunning and decapitation. NAC and DS tissue samples were taken from a 1 mm-thick tissue slice at a distance of 5.5 mm from the frontal pole using established procedures (17).

Tissue samples from each rearing group were divided into two groups: basal and DA-stimulated groups. The procedure used for tissue preparation and analysis has been previously described (17). All samples were analyzed by a prelabelling technique wherein the samples were exposed to [^{3}H]-adenine (26 Ci/mMol) for 40 min. After this prelabeling, sulpiride (50 μM), a D_2 receptor antagonist, was added to all the samples, which were incubated for 20 min at 37°C to allow equilibration. DA (100 mM) was then added to half of those samples and incubated for a further 20 min. Sequential Dowex-alumina ion-exchange chromatography was then used to isolate the [^{3}H]-cAMP, and radioactivity was determined by liquid scintillation counting.

Measurement of [^{3}H]-cAMP Accumulation in the NAC Following DA Stimulation With and Without Sulpiride

Samples from the NAC were prepared and analyzed as in the previous experiment. The samples from each rearing group (n = 12 ISO, n = 12 SOC) were divided into four groups: basal and stimulated, with or without sulpiride. The samples were exposed to [^{3}H]-adenine (26 Ci/mmol) for 40 min. After this prelabeling sulpiride (50 μM) was added to the half the samples and incubated for 20 min at 37°C to allow equilibration. DA (100 mM) was then added to half of the samples in each group and incubated for a further 20 min. As in the previous experiment, sequential Dowex-alumina ion-exchange chromatography was used to isolate the [^{3}H]-cAMP and radioactivity was determined by liquid scintillation counting.

Data Analysis

The D_1 receptor function data, based on cAMP accumulation in nucleus accumbens and dorsal striatum following DA stimulation in the presence of sulpiride, were analyzed by a four-way ANOVA with between-subjects factors of rearing, brain region (NAC vs. DS), STIM (basal vs. DA stimulated), and side (right vs. left). The data on NAC D_1 and D_2 receptor function, based on cAMP accumulation in the NAC in the presence or absence of sulpiride, were analyzed by a four-way ANOVA with between-subjects factors of rearing, presence of sulpiride, STIM, and side. Stereotypy and activity data were submitted to ANOVA with the between-subjects factors of rearing and drug (saline vs. apomorphine) and the repeated measure of time.

RESULTS

[^{3}H]-cAMP Accumulation in Nucleus Accumbens and Dorsal Striatum Following DA Stimulation in the Presence of Sulpiride

D_1 receptor activation by 100 μM DA in the presence of sulpiride produced a significant increase in cAMP accumulation in both NAC and DS tissue slices, $F(1, 32) = 115.5$, $p < 0.004$ (Fig. 7A). In the NAC, cAMP accumulation increased in SOC subjects by 59% and in ISO subjects by 71%. In the DS, the increase in SOC rats was 145% and in ISO rats was 155%. In both groups cAMP levels were consistently higher in the DS compared to the NAC, $F(1, 32) = 28.23$, $p < 0.004$. The percent increase in cAMP levels due to D_1 stimulation was also higher in the DS compared to the NAC, $F(1, 32) = 25.6$, $p < 0.004$. Rearing was without effect upon cAMP levels under any condition.

[^{3}H]-cAMP Accumulation in the NAC Following DA Stimulation With and Without Sulpiride

There were no differences in nonstimulated cAMP levels in the NAC between ISO and SOC rats. As in the previous experiment 100 mM DA under sulpiride produced a substantial increase in cAMP levels in both groups, $F(1, 32) = 20.5$, $p < 0.004$ (Fig. 7B). There was no difference in the magnitude of

this increase due to rearing. In ISO rats cAMP levels increased by 74% and by 76% in SOC rats. The accumulation of cAMP produced by 100 mM DA without sulpiride was reduced in both rearing groups, compared to the accumulation in the presence of sulpiride, as demonstrated by a significant interaction between STIM and sulpiride, $F(1, 32) = 8.16$, $p < 0.008$. The absence of sulpiride in the media reduced cAMP to basal levels in the SOC rats but in ISO subjects cAMP levels remained 31% above baseline. This D_2 receptor downregulation was confirmed by a significant interaction between rearing and sulpiride, $F(1, 32) = 5.75$, $p < 0.03$, although the interaction between rearing, sulpiride, and STIM failed to reach significance, $F(1, 32) = 2.80$, $p < 0.10$.

Apomorphine-Induced Stereotypy and Locomotor Activity

Apomorphine administration produced pronounced stereotypy in both rearing groups, as measured using a five-point stereotypy scale, $F(1, 12) = 14.05$, $p < 0.003$ (data not shown), although there was no effect of rearing on stereotypy. In terms of activity however, SOC rats had lower activity scores than ISO rats under all conditions, $F(1, 12) = 7.74$, $p < 0.02$. Apomorphine decreased activity in the SOC group compared to saline but did not do so in ISO subjects. This was confirmed by a significant interaction between rearing, drug, and time, $F(11, 132) = 2.02$, $p < 0.04$. Thus, ISO rats were less sensitive to the activity decreasing effects of apomorphine than SOC rats.

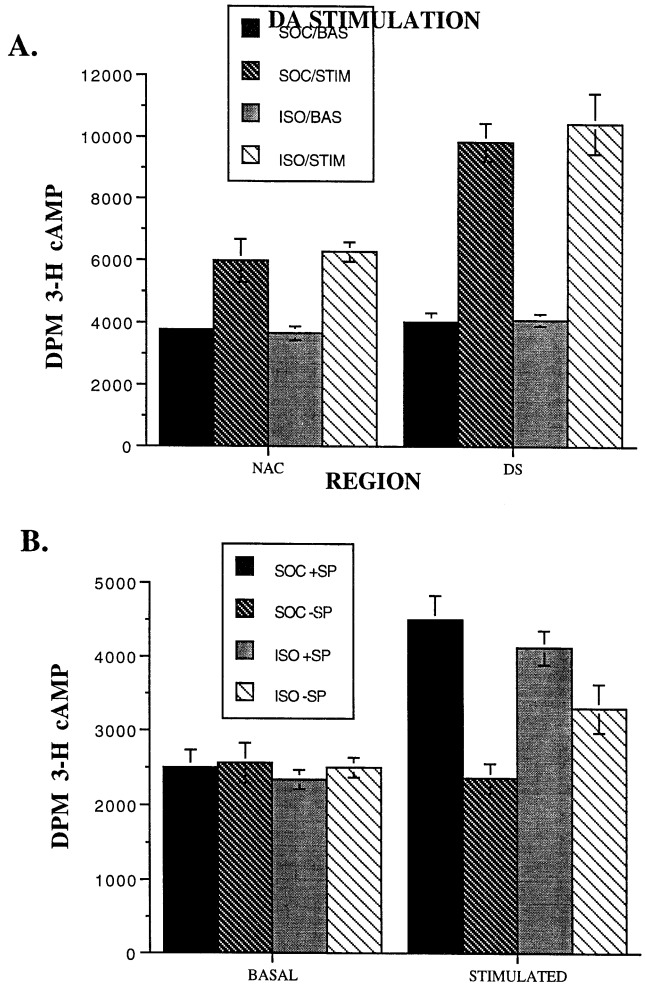

FIG. 7. Postsynaptic effects of isolation rearing from Experiment 3. The effect of 100 mM DA (STIM) or no DA (BAS) on [^{3}H]-cAMP accumulation in the NAC and CP in isolation-reared (ISO) and socially reared (SOC) rat was unaltered (A). Basal and stimulated [^{3}H]-cAMP accumulation in the presence of sulpiride (+SP) or without sulpiride (−SP), ISO, and SOC rats in the NAC (B). D2 inhibition was impaired in isolation-reared rats.

DISCUSSION

The attenuation of the effect of removing sulpiride on dopamine-induced cAMP production in slices of nucleus accumbens taken from isolates is consistent with a functional downregulation of dopamine D_2 receptors in this brain area. This effect is consistent with the effects of apomorphine on isolation-reared rats (17) and also the effects of raclopride on amphetamine self-administration in isolation-reared rats (26).

D_1 receptor function in the DS and NAC in this study, defined in terms of cAMP accumulation in tissue slices, are consistent with previous reports (21,23). There were no differences in D_1 receptor function between rearing groups in response in the CP, consistent with a previous study (17). Furthermore, there were also no differences in D_1 receptor function in the NAC between ISO and SOC rats in Experiment 3. Inhibition of cAMP accumulation by D_2 stimulation by dopamine in the absence of sulpiride in the NAC is consistent with some findings (23), but not with others (21). The reasons for these discrepancies are not readily apparent, but the relationship between stimulation of dopamine receptor subtypes and G-protein function appears to be an intrinsic property of each receptor that does not vary regionally (35). However, the present findings suggest that these discrepancies might possibly be the result of differing housing conditions of the subjects that were not fully documented in any of these previous biochemical studies.

GENERAL DISCUSSION

The results of these experiments clearly demonstrate that isolation rearing of rats increases presynaptic DA function in the NAC, as found previously (17,40). The neurochemical effects of *d*-amphetamine, confirmed in Experiments 1 and 2, clearly concur with the behavioral differences observed after amphetamine administration (17,19). Furthermore, there were also effects of another early-life manipulation—handling/repeated testing.

Repeated Behavioral Testing/Handling

Two important effects of repeated handling/testing were demonstrated in Experiment 1. First, repeated handling/testing had effects on some of the same behavioral and neurochemical systems that were also affected by isolation rearing. Second, not all of these effects were identical, which would rule out the possibility that the effect of both stressors could be accounted for by the same general mechanism.

Repeated handling/testing reversed both the hyperactivity of isolation-reared rats and increased weight gain compared to nontested subjects. However, in contrast to these results, repeated behavioral testing produced increases in basal DA efflux and the response to *d*-amphetamine. Thus, the same ISO RH rats that had greater basal extracellular levels of DA and increased responses to *d*-amphetamine, were the same rats that were no longer hyperactive compared to equivalently

treated SOC RH RATS after 13 testing sessions. In the same way, only ISO NH rats exhibited the increased weight-gain characteristic of isolation-reared rats.

Supporting the idea that different mechanisms were involved in the effects of these two chronic treatments, the DOPAC response to *d*-amphetamine was altered by repeated handling/testing but not isolation rearing. Therefore, increased DA efflux may be the result of alterations in different mechanisms regulating DA release. Finally, the changes in 5-HIAA levels were affected in opposite directions by handling and isolation rearing. An important implication of these findings is that in experiments that necessitate extensive handling/testing, some effects of isolation rearing might be diminished, while others might be exaggerated.

This study has also revealed that repeated handling/testing of weanling rats alone has profound and enduring effects on the dynamics of DA release in the NAC. These effects, moreover, are in the same direction as those occasioned by isolation rearing. Such an observation has important implications for those studies in which, during early postweaning, subjects received repeated handling/testing. The key point is it may be difficult to discern effects due to handling/testing from those due specifically to isolation rearing. It should be emphasized, however, that these data do not provide any information about the effects of repeated handling/testing or social isolation in mature animals.

The Nature of the Effects of Isolation Rearing on Dopamine Function

The behavioral syndrome produced by isolation rearing, including the hyperactivity replicated in the present experiments, is consistent with the increased basal levels of DA observed in Experiment 1. Although only a trend toward higher basal DA levels was found in Experiment 2, because there were so many subjects, tested over an extended period of time, it is possible that such an effect may have been masked. As suggested, a number of changes in DA neurons might be relevant to these effects, including changes in terminal density, rate of neuronal discharge, neurotransmitter reuptake, autoreceptor regulation, and presynaptic modulation by other neurotransmitters. For instance, Hall et al. (15) found that isolation-reared rats have increased levels of tyrosine hydroxylase in the nucleus accumbens shell. However, contrary to this result, decreased dopamine terminal density was found in the striatal matrix of isolated monkeys (22). However, these monkeys had been both peer deprived and maternally deprived.

The precise mechanism by which isolation rearing enhances presynaptic DA function could not be definitively determined in the present experiments. However, the results of Experiment 2 suggest that increased density of DA innervation alone cannot account for this effect. If that were the case, increased local K^+ and *d*-amphetamine would be expected to have the same effect on extracellular DA levels, which was not the case. Several studies have failed to find differences in NAC tissue levels of DA following isolation rearing (17,18,37,38), also suggesting that increased levels of extracellular DA in isolates are not the result of increased DA innervation. Regardless of the underlying mechanism of isolation-induced changes in DA systems, the in vivo microdialysis results clearly demonstrate the deficiencies of postmortem brain analyses that have failed to demonstrate effects of isolation rearing on DA function at all.

It would seem apparent that the difference between isolation-reared rats and socially reared rats in presynaptic response to *d*-amphetamine must be attributed to some factor that *d*-amphetamine acts on uniquely (such as a greater density of reuptake sites), or perhaps the interaction between other NAC afferents and DA terminal release that is differentially affected by *d*-amphetamine and potassium.

Autoreceptor downregulation might also play a role in increased DA function in isolation-reared rats observed in the present experiments. Autoreceptor specific doses of DA agonists were less effective in producing penile erections, stretching, and yawning in isolation-reared rats (8). In contrast, the hypoactivity induced by low doses of apomorphine was enhanced in isolation-reared rats (17). However, such effects cannot explain the difference between the effects of *d*-amphetamine and K^+ on isolation-reared rats.

The actions of K^+ on other NAC afferents that also affect DA release might explain the differences between the effects of *d*-amphetamine and K^+. The results of some studies might be taken to indicate changes in these other NAC afferents. For example, isolation-reared rats have been shown to have deficits in prepulse inhibition (12,40). Not only is prepulse inhibition impaired by dopamine agonists, but also by the NMDA antagonists PCP and MK801 (11). This suggests the possibility at least that glutamate mechanisms may be involved in impairments of prepulse inhibition and changes in presynaptic dopamine function in isolation-reared rats.

Further complicating interpretations at the behavioral level of the presynaptic effects of isolation rearing, Experiment 3 demonstrated a downregulation of postsynaptic dopamine D_2 receptors in the NAC. It has previously been shown that isolation rearing does not affect cAMP responses to D_1 or D_2 stimulation in the dorsal striatum (17). Those authors suggested that the lack of changes in postsynaptic DA receptor function in the dorsal striatum might account for the greater consistency in the effects of amphetamine compared to apomorphine (2). Because isolation rearing increased DA release in both the NAC and dorsal striatum (17) but D_2 receptors were downregulated only in the NAC the actions of direct DA agonists should be greater on behavior mediated by the NAC in isolates relative to socially reared rats. The apomorphine results of Experiment 3 agree with this hypothesis, as do the effects of D_2 antagonism on self-administration (26). What is harder to explain is why low doses of the D_2 receptor antagonist raclopride can reverse deficits in prepulse inhibition in isolates (12) unless the net effect of enhanced presynaptic DA release with reduced postsynaptic D_2 receptor function is to increase DA functions.

Depending on the final outcome of these changes in presynaptic and postsynaptic DA function in the NAC, isolation-reared rats might be expected to have greater DA efflux in response to stimuli that increase DA cell firing, such as novel stimuli (34). The prototypical hyperactivity of isolates is dependent on environmental novelty (10). Isolation-reared rats also show enhanced response to novel objects (29), enhanced preference for a novel environment (14,29), and increased environmental neophobia in an open field (13). The universality of such behavioral changes might depend on the interactions between different isolation-induced neurochemical alterations.

Strain Differences in Response to Isolation Rearing

A previous behavioral study (17) reported increased sensitivity to systemic *d*-amphetamine in isolation-reared rats at lower doses. The failure to find differences between rearing groups at lower doses of *d*-amphetamine in Experiment 2 may have been due to a number of factors, but one interesting possibility is differences in the strain of the subjects compared to previous experiments (17,40).

Comparing Experiments 1 and 2 it seems that, regarding strain differences in isolates, three conclusions may be drawn: 1) Sprague–Dawley rats have lower basal DA levels than Lister hooded rats; 2) Sprague–Dawley rats have a smaller response to *d*-amphetamine than Lister hooded rats; and 3) for some reason, which may very well have to do with points 1 and 2, Sprague–Dawley rats are less sensitive to the effects of isolation rearing than Lister hooded rats. Thus, the sensitivity to isolation rearing in rats may vary with genetic background. However, these crossexperiment comparisons must remain speculative until the interaction between strain and isolation rearing is examined explicitly.

FINAL CONCLUSIONS

These experiments have replicated previous findings demonstrating presynaptic DA changes subsequent to isolation rearing of rats, and suggest that postsynaptic changes occur as well. More broadly, they support the involvement of DA systems in the effects of chronic stressors (repeated handling/testing and isolation rearing) more generally, although differences in the precise effects produced by each manipulation were observed. Although these experiments could not identify the underlying mechanisms of increased presynaptic DA function in isolation-reared rats, it appears that there may be multiple changes, perhaps in the interaction between DA terminals and other NAC afferents.

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