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Reactivity to Amphetamine in Perinatally Undernourished Rats: Behavioral and Neurochemical Correlates

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BRIONI, J D , E A KELLER, L E LEVIN, N CÓRDOBA AND O A ORSINGHER *Reactivity to amphetamine in perinatally undernourished rats Behavioral and neurochemical correlates* PHARMACOL BIOCHEM BEHAV 24(3) 449–454, 1986 —Adult rats deprived at perinatal age and then rehabilitated on balanced chow were treated with a multiple amphetamine (AMPH) schedule (2 mg/kg/48 hr) and submitted, on days of injections, to an open-field test Throughout 11 sessions, deprived rats showed a progressive increase of locomotor activity as compared with controls Stereotyped activity evaluated during the AMPH treatment did not differ between control and deprived animals No differences were detected in basal values of the dopaminergic function measured in naive control and deprived animals By the end of the multiple AMPH treatment, a reduction of striatal DA and DOPAC levels together with a lower apparent DA turnover rate was detected in deprived animals Besides, DA receptor binding was significantly increased in striatum from deprived rats as compared with controls These results demonstrate that a repeated AMPH treatment, that was unable to alter the normal behavior of control rats, produced in early undernourished animals a progressive sensitization to AMPH effects, in addition to significant changes in the striatal dopaminergic function

Early undernutrition Multiple amphetamine administration Locomotor activity Stereotypy Dopaminergic function

PROTEIN malnutrition in early life produces deleterious effects on different anatomical, neurological and neurochemical parameters (see Morgane *et al* [15]) as well as behavioral alterations [13, 17, 23] that persist throughout adulthood Changes in the levels and metabolism of brain biogenic amines were also described [9, 22, 24, 25, 27] Recently, Hall *et al* [8] reported that adult rats undernourished in early life showed a reduced sensitivity to the central effects of serotonergic agonists, probably as a consequence of an altered metabolism of this neurotransmitter As regards the catecholaminergic system, it was described that perinatally deprived rats later on rehabilitated on balanced chow for at least 90 days, showed both an increased turnover rate of brain dopamine (DA) and noradrenaline (NA) and an enhanced tyrosine-hydroxylase activity [14] This higher neuronal activity induces a significant reduction in the number of alpha and beta adrenergic binding sites [11]

In order to study the functional significance of the alterations in the central catecholaminergic system induced by early undernutrition, we studied the reactivity to amphetamine (AMPH) during a multiple administration schedule on different behavioral parameters measured in an open-field and on the induction of stereotyped activity On the other hand, with the purpose of correlating behavioral and neurochemical changes, endogenous levels of DA and its deaminated metabolite DOPAC, DA turnover rate and dopaminergic receptor binding in striatum and accumbens nucleus from control and experimental animals were also assayed

METHOD

Animals

A protein deprivation schedule as previously described was used [14] Pregnant female rats, derived from Wistar strain, were divided into two groups at the 14th day of pregnancy, housed in individual Plexiglas cages and fed isocaloric diets containing 24 and 8 percent casein (control and deprived, respectively) After weaning (24 days), groups continued consuming the same diet as their dams until the end of the deprivation period (50 days of age). Thereafter, both groups were given balanced standard chow for at least 90 days prior to experiments Animals were maintained at 22±2°C in a 12 hr-12 hr light-dark cycle (light beginning at 7 00 hr a m) Food and water were available ad lib

Drugs

D-L amphetamine phosphate (kindly supplied by Laboratories Lazar & Co —Buenos Aires) was dissolved in saline

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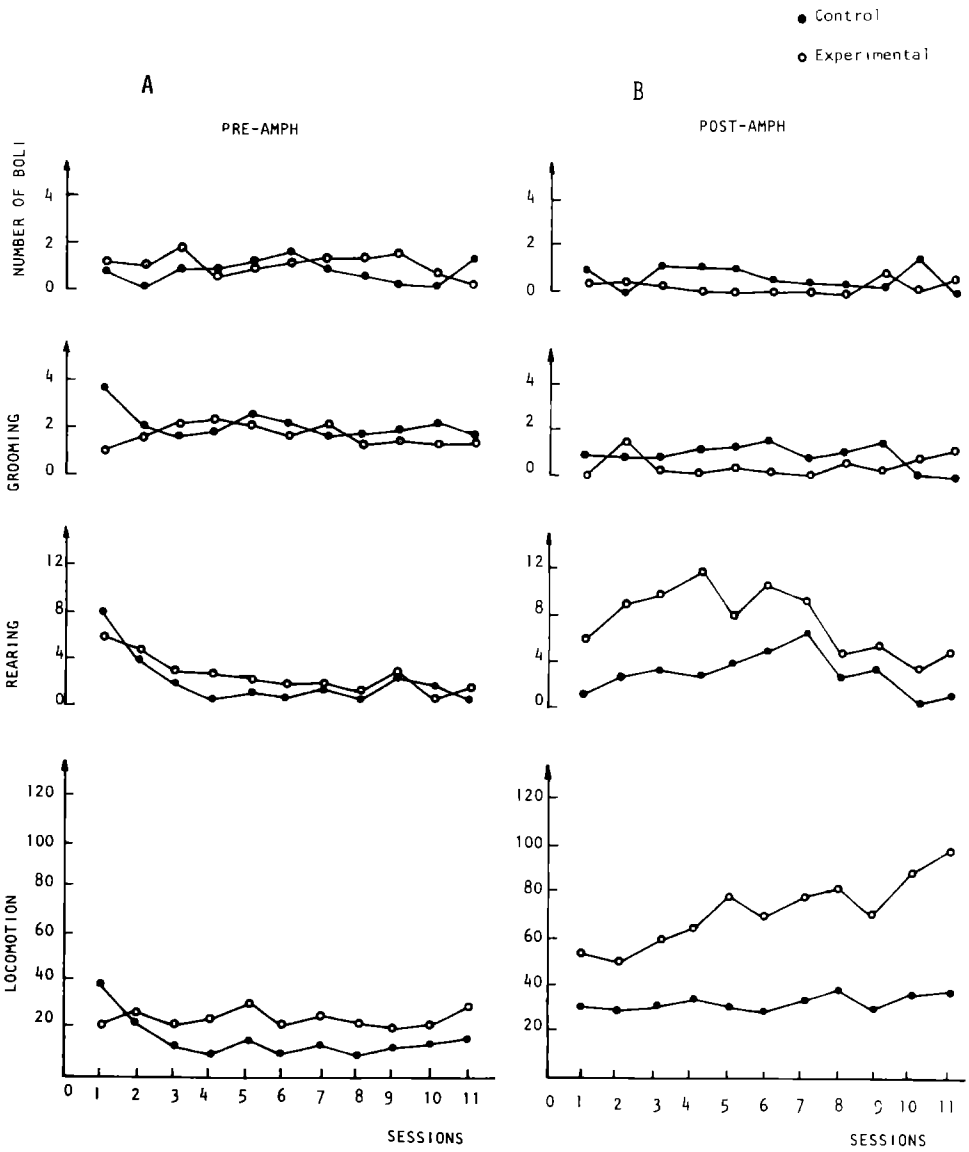


FIG 1 Open-field scores during repeated AMPH injections separated by 48 hr intervals (A) Pre-AMPH scores Animals were tested 15 min after saline injection Note the lack of habituation in locomotion from deprived rats as compared with controls (B) Post-AMPH scores recorded following basal tests, 15 min after the injection of AMPH (2 mg/kg IP) In experimental animals, scores of locomotor activity were higher than controls and there was also an increased reactivity throughout the treatment

for IP injections Dosages were expressed as salt D-L α-methyl-p-tyrosine (Sigma Chemical Co.) was suspended in saline and Tween-80 for IP injections

Behavioral Studies

Open-field A square open-field (60×60×60 cm) was used Its floor was painted gray and divided into 15×15 cm squares by black lines. Tests were performed in a darkened room and the box was uniformly lit from above by a 75 W bulb The following parameters were determined locomotion (numbers of squares crossed with all four feet), rearing on hind legs (with or without leaning against the wall), grooming and number of boli emitted Scores were recorded by two observers

Procedure Twelve male rats from each group (control and experimental) were allowed for 7 days into the test-room before beginning the experiment Each animal was removed from the cages, placed in the center of the open-field and submitted to a double open-field session of 3 min each The first test was performed 15 min after saline administration to obtain the pre-drug baseline activity (pre-AMPH scores), thereafter, the rat was immediately injected with AMPH (2 mg/kg IP) and 15 min later subjected to the second open-field test (post-AMPH scores) The multiple AMPH administration schedule was achieved by repeating the double open-field sessions every 48 hr during 21 days (i e , 11 sessions) Experiments started at 8 30 a m

Data were analyzed using two-way analysis of variance

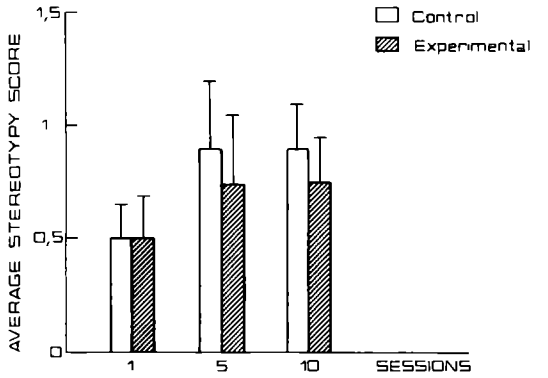
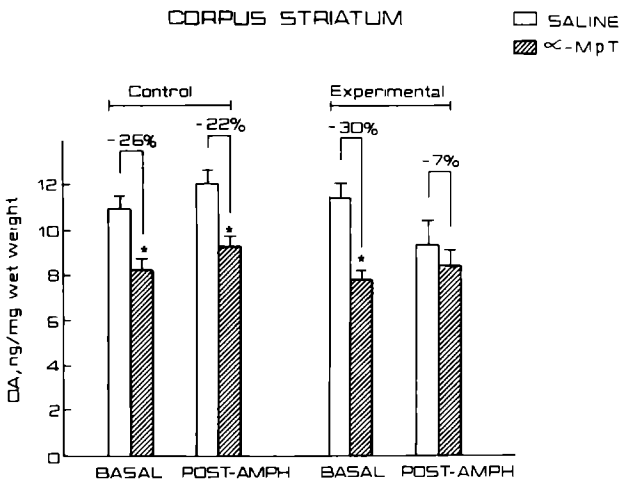


FIG 2 Evaluation of stereotyped activity on sessions 1, 5 and 10 of the multiple AMPH treatment Stereotypy was estimated every 5 min during 30 min, according to Naylor and Costall (range 0–4) Each bar represent the mean±SEM from 7 rats No difference was detected between control and deprived rats in any session



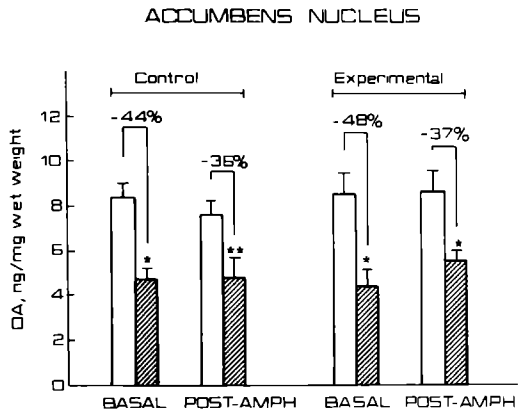


FIG 3 Decline in DA levels following α-methyl-p-tyrosine (200 mg/kg IP), before and after the multiple AMPH treatment Animals were killed 2 hr after the inhibitor injection for DA determinations Synthesis inhibition induced significant reduction in DA levels from all groups at exception of the striatal post-AMPH deprived group In this group the lower reduction of DA levels together with lower "0" time values, suggests a lower DA turnover rate $*p<0.01$, $**p<0.02$ against "0" time values by two tailed Student's t-test

after square root transformation, followed by individual comparisons using the Mann-Whitney "U" test

Evaluation of stereotypy Two groups (control and experimental) of 7 male rats each were subjected to a repeated AMPH treatment similar to that used for the open-field experiment (2 mg/kg/48 hr) On days corresponding to sessions 1, 5 and 10 (days 1, 9 and 19), rats were individually placed in a wire mesh cage (40×40 cm) immediately after AMPH injection and monitored for stereotyped behavior during 30 min The intensity of stereotypy was assessed every 5 min according to the scoring system of Naylor and Costall [16]

Neurochemical Assays

Different aspects of the dopaminergic function were studied in striatum and accumbens nucleus from naive control and experimental rats, and in animals treated with the multiple AMPH administration schedule as used in behavioral experiments

DA and DOPAC assays Endogenous levels of DA and DOPAC were determined in striatum and accumbens nucleus from naive animals (basal levels) Post-AMPH levels were determined at the time of the last AMPH injection (i e , 48 hr after the 10th AMPH injection) DA and DOPAC were measured by HPLC with electrochemical detection [28] Briefly, brain structures were removed according to Heffner *et al* [10] and homogenized in 0.2 N perchloric acid After centrifugation, the supernatant was passed through a 0 2 μm pore size cellulose filter and a 20 μl sample analyzed by HPLC/ED (Bioanalitical Systems IN) Samples were chromatographed on a reverse-phased ion paired column containing microparticulate bound C18 as the stationary phase and 0.05 M sodium acetate (pH 4) containing 1 5% methanol as mobile phase Retention times were 4 min for DA and 11 min for DOPAC. Mean values obtained were analyzed by Student's t-test

Estimation of apparent DA turnover rate The decline in DA levels following synthesis inhibition by α-methyl-p-tyrosine was used as an index of DA turnover rate [1] Naive animals from both groups were killed 2 hr after the injection of the inhibitor (200 mg/kg IP) Saline-injected rats were used to determine basal levels that served as "0" time point A similar procedure was employed for AMPH treated rats 48 hr after the 10th AMPH injection (corresponding to the time when endogenous levels of DA and DOPAC were analyzed)

DA receptor binding assay Dopaminergic receptor binding was performed in striatum from naive rats (control and deprived), and in those animals used in the open-field experiments, 24 hr after the last session ^{3}H-Spiroperidol binding was carried out according to Creese *et al* [3] Tissues were homogenized in 100 vol of ice-cold 50 mM Tris-HCl buffer (pH 7 4 at 37°C) and homogenates were twice centrifuged at 50,000 g for 10 min with rehomogenization of the intermediate pellet in fresh Tris buffer Final pellets were suspended in 50 mM Tris buffer containing 0 1% ascorbic acid, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$ (pH 7 1 at 37°C) to get a final concentration of 2 0 mg wet weight/ml Incubations were carried out by triplicate at 37°C for 15 min; membrane bound ^{3}H-Spiroperidol separated by rapid filtration

TABLE 1

REGIONAL DA AND DOPAC CONCENTRATIONS IN CONTROL AND UNDERNOURISHED RATS

	Basal		Post-AMPH‡	
	DA	DOPAC	DA	DOPAC
Striatum				
Control	11.1 ± 0.5	1.0 ± 0.1	12.1 ± 0.5	1.1 ± 0.06
Undernourished	11.4 ± 0.8	1.1 ± 0.1	9.4 ± 1.0*	0.9 ± 0.08†
Accumbens Nucleus				
Control	8.3 ± 0.9	1.3 ± 0.1	7.6 ± 0.6	1.4 ± 0.2
Undernourished	8.2 ± 0.5	1.4 ± 0.1	8.7 ± 0.8	1.4 ± 0.2

*$p<0.05$, †$p<0.01$ by Student's *t*-test

‡Animals were killed at the time corresponding to the last open-field session (i.e., 48 hr after the 10th AMPH injection)

Results are mean ± SEM in ng/mg wet weight tissue of 5–7 animals

TABLE 2

^{3}H-SPIROPERIDOL BINDING IN STRIATUM FROM CONTROL AND UNDERNOURISHED RATS

	Basal		Post-AMPH‡	
	Kd nM	Bmax pmol/gww	Kd	Bmax
Control	2.3 ± 0.6 (5)	15.8 ± 1.1 (5)	1.8 ± 0.2 (9)	16.7 ± 1.2 (9)
Undernourished	2.4 ± 0.6 (5)	18.4 ± 1.1 (5)	2.0 ± 0.2 (9)	24.2 ± 1.2* (9)

*$p<0.05$ by Student's *t*-test

‡Animals were killed 24 hr after the 11th AMPH injection

Results are mean ± SEM. The number in parentheses represent the number of independent determinations. Bmax were calculated from Scatchard plots and expressed as pmol/g wet weight tissue

under vacuum over Whatman GF/B filters and washed three times with ice-cold Tris buffer. Filters were dried and transferred to vials containing Triton X-100, toluene and PPO for counting radioactivity into a liquid scintillation counter. Stereospecific binding was defined as the difference in binding in the presence and absence of 1 μM d-butaclamol. The apparent dissociation constant (K_d) and maximal binding capacity (B_{max}) were calculated from saturation curves using six concentrations of ^{3}H-Spiroperidol (0.1–5.0 nM). Scatchard plots were obtained by least square regression analysis. K_d and B_{max} values were statistically analyzed by Student's *t*-test.

RESULTS

Open-Field Behavior

Figure 1 A shows the pre-AMPH scores obtained in the different behavioral parameters studied in the open-field. No differences between control and experimental rats were detected in the number of boli, grooming or rearing. As regards locomotor activity, in the control group there was a significant session effect, $F(10,241)=3.24$, $p<0.025$, as control rats developed the classical habituation phenomenon (session 1 vs session 11, $U=3$, $p<0.002$). Habituation was absent in deprived rats which maintained a similar score throughout the sessions (session 1 vs session 11, $U=48$, N.S.). Lack of habituation to locomotion in deprived rats was also present when the open-field experiment was repeated with other group of rats that only received saline injections in each session (data not shown).

Post-AMPH scores are shown in Fig. 1 B. Number of boli and grooming scores did not differ between both groups. Deprived rats showed a higher rearing response, $F(1,242)=35.4$, $p<0.005$. When analyzed individually using Mann-Whitney "U" test, differences between control and deprived rats were found on the 2nd, 3rd and 4th sessions (all U's, $p<0.05$). On the remaining sessions the differences were not significant. Locomotor response to AMPH was significantly greater in deprived animals as compared to controls, $F(1,241)=153$, $p<0.005$, and they also showed a gradually enhanced reactivity to repeated drug administration (session 1 vs session 11, $U=19$, $p<0.002$). The score of control rats was not affected with a new challenge dose throughout the open-field experiment (session 1 vs session 11, $U=47$, N.S.).

When the difference in locomotion between pre and post drug tests was analyzed between both groups, a significant difference was observed, $F(1,240)=124$, $p<0.005$. These results indicate that the enhanced reactivity to AMPH was independent of the elevated basal locomotor activity of deprived animals.

Stereotyped Behavior

Figure 2 depicts the average stereotypy scores induced by

AMPH in sessions 1, 5 and 10, during the multiple AMPH treatment The intensity of stereotypy was similar between control and deprived rats, since both groups displayed a constant locomotor activity with discontinuous sniffing

Neurochemical Determinations

DA and DOPAC assays Basal levels of DA and DOPAC in striatum and accumbens nucleus determined in naive control and experimental animals, did not differ between them On the contrary, repeated AMPH administration produced a significant reduction in striatal DA and DOPAC levels from deprived animals as compared with controls No differences between both treated groups were detected in accumbens nucleus (Table 1)

DA Turnover Rate

The apparent DA turnover rate in striatum and accumbens from naive control and experimental rats was similar The multiple AMPH treatment induced a significant reduction in striatal DA turnover rate from deprived animals as compared with controls No difference was detected in accumbens nucleus after repeated AMPH treatment between both groups (Fig 3)

DA Receptor Binding

Table 2 indicates that the basal values of K_d and B_{max} of striatal dopaminergic receptors determined in naive control and deprived animals were not different The repeated AMPH treatment did not modify these kinetic parameters in the control group On the contrary, by the end of the AMPH treatment, deprived rats showed a significant increase in B_{max} without changes in affinity

DISCUSSION

AMPH facilitates catecholaminergic (CA) neurotransmission by promoting CA release from nerves terminals and by blocking presynaptic CA uptake [2, 5, 7] In addition, AMPH inhibits MAO [6] Different reports have demonstrated that repeated administration of AMPH induces a progressive increased response to some behavioral effects, particularly locomotor and stereotyped activities [20,21] At present, no conclusive evidence about the mechanism of this hypersensitivity phenomenon has been obtained

Since early undernutrition provokes alterations in the central catecholaminergic system [11,14], that may affect neuronal activity, it seemed interesting to investigate the reactivity of deprived animals to a multiple AMPH administration schedule on the open-field behavior and on the induction of stereotyped activity

In the open-field test, pre-AMPH scores showed no difference between control and experimental animals on the parameters measured, with the exception of locomotion The control group developed habituation from the onset of the experiment, an effect not observed in the deprived group, which maintained a constant score throughout the experiment The possibility that the lack of habituation in deprived rats was due to conditioned drug effects [26] may be ruled out, as this effect was also present in open-field experiments conducted with rats only injected with saline

It is possible that the lack of habituation to locomotor activity exhibited by deprived animals may be a consequence of the heightened emotionality induced by early undernutrition In this regard many reports have pointed out alterations in some behavioral aspects related to emotionality, such as increased pre and postshock latencies to enter a shock chamber [23,24], increased immobilization to a loud noise [13], hyperactivity in a Sidman avoidance situation [13] and higher lever pressing rates in a variable-interval operant schedule [23]

Post-AMPH scores demonstrate that our schedule of treatment did not induce a progressively enhanced locomotor responsiveness to AMPH in control rats This may be due to the mild schedule of treatment used (2 mg/kg ±AMPH phosphate, expressed as salt, injections every 48 hr), since most of the authors that described this phenomenon used higher doses separated by shorter intervals [19, 20, 21] On the contrary, deprived animals showed higher rearing scores than controls up to the fourth session, while locomotion was higher and progressively increased throughout the sessions These different behavioral patterns of reactivity to AMPH between rearing and locomotion in the experimental group suggest that these activities may be competitive

As regards the stereotyped activity, the scores obtained demonstrate that the treatment used was unable to induce noticeable changes in either group, since according to Naylor and Costall [16] a clear stereotyped behavior can be detected when scores reach values of 2 or higher

Different aspects of DA function analyzed in brain areas related to the regulation of the behaviors studied revealed that in basal conditions there were no differences between both groups At the end of the multiple AMPH treatment, no significant changes in the control group were detected in any of the parameters analyzed On the contrary, in deprived rats, a significant reduction of DA and DOPAC levels together with a lower DA turnover rate was observed in striatum but not in accumbens nucleus

DOPAC determinations in striatum provide a useful index of the functional activity of dopaminergic neurons, reflecting the metabolism of DA which has been released and recaptured at the presynaptic side [18] The reduction in DOPAC levels is consistent with the diminished DA turnover rate, indicating that lower amounts of DA are released in the deprived group at the end of the multiple AMPH treatment As a consequence, ^{3}H-Spiroperidol binding was significantly higher in striatum from deprived animals

The increased number of dopaminergic receptors found in striatum from deprived animals after repeated AMPH injections keeps a good correlation with the higher scores of locomotor activity observed in these animals throughout the experiments The diminished DA turnover rate and the reduction of DA and DOPAC levels in such structure could be interpreted as the result of presynaptic adjustments intended to balance the neuronal hyperactivity induced by AMPH treatment

Since deprived treated rats showed an enhanced locomotor activity than controls, it may be accepted that they behave as normal rats submitted to a higher AMPH dosage If so, the changes in dopaminergic function from undernourished rats are in agreement with the observation of Segal *et al* [21], who reported that multiple daily AMPH injections, that induced behavioral augmentation, reduced brain catecholamines levels, including caudate DA

Our results extend previous reports that demonstrated changes in reactivity to pharmacological treatments as a consequence of early undernutrition, such as subsensitivity to adrenergic agonist on the vascular bed [4], and other sympathetic innervated organs [12], lower reactivity to the central effects of 5-HT agonist [8], enhanced sexual receptivity

in female rats ovariectomized and primed with estrogen and progesterone [17], that stresses the pathophysiological relevancy of an inadequate perinatal nutrition for a normal nervous system development

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