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Schedule-Induced Polydipsia: Gender-Specific Effects and Consequences of Prenatal Cocaine and Postnatal Handling

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KATOVIC, N. M., J. E. GRESACK AND L. P. SPEAR. *Schedule-induced Polydipsia: Gender-specific effects and consequences of prenatal cocaine exposure and postnatal handling.* PHARMACOL BIOCHEM BEHAV **64**(4) 695–704, 1999.—The impact of gestational cocaine in conjunction with postnatal handling on schedule-induced polydipsia (SIP) was examined. Rat offspring were derived from Sprague–Dawley dams injected subcutaneously with 40 mg/kg/3 cc cocaine hydrochloride (C40) on gestational days 8–20, dams injected with vehicle and pair fed 4 (PF4) days to mimic the acute anorexic effects of cocaine administration, and nontreated (NT) control dams. In adulthood, offspring were food deprived and given 13 daily 30-min SIP sessions, with water intake recorded during the scheduled (fixed time 60 s—FT60) food delivery. For 4 days thereafter, animals received saline, 5 or 10 mg/kg of cocaine in counterbalanced order prior to SIP testing. Acquisition and maintenance of SIP, but not cocaine-induced suppression of SIP performance, were observed to be dependent upon prenatal treatment, handling, and gender. Females acquired SIP faster and exhibited notably higher levels of polydipsia than males. Early handling increased levels of established SIP in NT offspring, while enhancing SIP acquisition in both PF4 and C40 offspring. In nonhandled animals, NT offspring exhibited less SIP than PF4 and C40 offspring, differences that were attenuated by early handling. These effects are discussed in relation to previously reported neurohormonal characteristics of these gender and treatment variables. © 1999 Elsevier Science Inc.

Schedule-induced polydipsia Early handling Prenatal cocaine exposure Gender differences Stress responsivity

SCHEDULE-INDUCED polydipsia (SIP) is the term used for excessive drinking that occurs in food-restricted animals when small amounts of food are presented intermittently, and free access to water is available (16,47,48). Although food restriction normally leads to a decrease in water intake, the increase in drinking associated with SIP develops across days in food-deprived animals as they are given repeated exposures to a scheduled food delivery of typically fixed time 60 s (FT60). It has been proposed that SIP is a displacement activity serving to help the food-restricted animal cope with the stressful situation of food deprivation and scheduled food delivery (5), or that it functions to reduce the state of arousal elicited by the motivational excitement that remains following consumption of the small food reinforcement (13,26). Indeed, levels of both the stress-sensitive glucocorticoid, corticosterone, as well as the neurotransmitter dopamine (DA) in stress-sensitive brain regions, have been shown to be related to the manifestation of this behavior.

Animals vary in the amount of SIP they develop (39), with levels of SIP being inversely related to postsession plasma corticosterone levels (37). Moreover, animals displaying SIP have significantly lower plasma corticosterone levels immediately following a session compared to presession levels (5). Mittleman and colleagues (36) found that the effects of corticosterone manipulations on SIP depend on whether the behavior is being acquired or is already well established, as well as the level of SIP that is displayed and the dose of corticosterone that is administered. Only a high dose of corticosterone is effective in increasing SIP in animals displaying high levels of polydipsia, while a low dose is effective in animals displaying low levels of polydipsia (37). When adrenalectomized (ADX) male rats are exposed to the SIP paradigm, the acquisition of polydipsia is inhibited, while corticosterone replacement in ADX rats reinstates the development of SIP (29). However, ADX does not disrupt SIP once high levels of this behavior are well established (36). Similarly, lesions of

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the mesolimbic DA system disrupt the development of SIP, but not regulatory eating and drinking (40,44); yet once high levels of SIP are established, DA-depleting lesions do not block this behavior (45). The effects of DA activity on SIP may constitute an inverted U-shaped function, with not only mesolimbic DA lesions (44), but also administration of DA agonists such as amphetamine suppressing SIP (45). Together, these data suggest that that the manifestation of SIP is related to endogenous levels of corticosterone and DA stimulation, with the occurrence of this displacement activity serving to alter subsequent neurohormonal activity.

These and other findings have led researchers to investigate individual differences that exist between animals and their propensity to develop SIP. Animals that display a high locomotor response to novelty (termed high responders—HR) show an increased corticosterone response when placed in a novel environment (15) and develop high levels of SIP (43), in comparison with animals that display low levels of locomotion in response to novelty (low responders—LR). Interestingly, when these animals are again tested for responsiveness to novelty after SIP exposure, HR rats display low levels of locomotor activity, while LR rats conversely display high levels of locomotor activity (43). Prior exposure to the SIP paradigm also has been found to attenuate polydipsia in animals exposed to the paradigm a second time (58). These findings suggest that an animal's propensity to develop polydipsia may be due to individual differences in such factors as its corticosterone response to stress, its prior experience, as well as its ability to cope with the stress invoked by the SIP paradigm itself. Among the factors that may contribute to these individual differences are gender of the organism and its developmental history.

Sex differences in corticosterone responses to stress have been widely documented. Female rats exhibit greater increases in plasma corticosterone levels than males when exposed to a variety of stressors including ether (27), footshock (57), forced running (25), and restraint (1), and are sometimes reported to exhibit elevated basal corticosterone levels as well (1,57). Twenty-four hours following exposure to a 2-h restraint stress, open-field activity of female rats is significantly less affected than that of male rats, while male rats show adaptation following repeated exposures and females do not (14). Gender differences in DA function are also evident. For instance, gonadectomized female rats have significantly lower basal release of DA in mesolimbic brain regions than their male counterparts, while showing enhanced amphetamine-stimulated DA release in striatum relative to castrated males (9). Despite the apparent relationship between levels of corticosterone, DA and SIP behavior observed from within gender comparisons and evidence for significant gender differences in these neurobehavioral measures, gender differences in SIP have not been systematically explored, with most investigations of SIP conducted in male rats [although females alone have occasionally been used; e.g., (17)].

Early developmental experience is another variable that has been shown to influence an animal's later ability to cope with stress, although few investigators have examined the impact of early experience on the later development of SIP. Jones and colleagues (24) found that rearing in isolation from weaning attenuated the development of SIP, effects that were not evident following a comparable period of isolation housing in adulthood. This effect may be related to previously observed effects of isolation rearing on functioning in stressor-sensitive dopamine (DA) systems (3,22). Another well-characterized early experience is "handling," a procedure consisting of isolating pups from the dam and their siblings daily for a short period of time early in ontogeny. Meaney and colleagues (34) have found that handling for 15 min daily for the first 7 days following birth is most effective in eliciting long-term alterations in hormonal stress responsivity. Adult animals exposed to early handling exhibit an attenuated plasma corticosterone response to various stressors when compared with nonhandled animals (28,33). Although baseline levels of plasma corticosterone generally are not different between handled and nonhandled animals (33), handled animals display a significantly faster poststress return to baseline levels compared to nonhandled animals (46). Effects of early handling on later SIP performance have not, to our knowledge, been previously investigated.

Taken together, this developmental literature suggests that early experiences influencing the ontogeny of the hypothalamic–pituitary–adrenal (HPA) axis as well as central DA systems may influence the animal's later ability to cope with stressful environmental events in adulthood. Another early manipulation reported to alter later dopaminergic activity (35) as well as later susceptibility to stressors and vulnerability to environmental demands in both clinical populations (31) and in rodent models [e.g., (53)] is that of gestational cocaine exposure. Adult animals prenatally exposed to cocaine have been reported to exhibit a reduced number of spontaneously active DA neurons in the substantia nigra and VTA (35) as well as long-term alterations in stress responsivity, including an attenuation in the immobility response induced by various stressful situations (4,41,53,59). Although basal levels of corticosterone and adrenocorticotropic hormone (ACTH) appear unaltered following prenatal cocaine exposure (2,6), the effects of prenatal cocaine exposure on corticosterone and ACTH reactivity are less conclusive, with evidence for more sustained stress-induced elevations of corticosterone (10) balanced by other data reporting an attenuated ACTH response to novelty when rat offspring are tested in adulthood (21) and lower cortisol responses to stressors in human exposed offspring tested in infancy (30). Preliminary data from our laboratory support the suggestion that cocaine-exposed offspring may also be differentially sensitive to the long-term effects of early environmental experience and that these differences are gender specific (53).

Thus, both prenatal cocaine exposure and early environmental experiences have been found to produce long-term changes in animal's ability to cope with stressors. These changes in stress responsivity have been suggested to be due to alterations in the HPA axis and/or stress-sensitive DA regions, physiological systems that have been shown to influence SIP behavior and whose activity is gender specific. Thus, the present study will explore the effects of prenatal cocaine exposure and early handling in male and female adult rats using the SIP paradigm. Additionally, following 13 days of SIP testing, dose-related suppressant effects of cocaine on SIP will be examined, given that prenatal cocaine exposure has been found to reduce sensitivity to cocaine in adulthood [e.g., (19)], and females tend to be more sensitive to stimulant administration than males (9). It is expected that females will display higher levels of SIP due to the fact that they typically have higher corticosterone levels and show greater behavioral responses to stressors compared to males. Early handling is conversely hypothesized to decrease levels of SIP, given that handled offspring as adults display lower peak levels of corticosterone following stressor exposure as well as a faster return to baseline. Finally, although the evidence is mixed regarding the effects of prenatal cocaine exposure on later HPA reactivity,

the compromised DA systems of these animals leads to the prediction that they will display lower levels of SIP compared to nontreated and pair-fed control offspring.

METHOD

Breeding Procedure

Offspring were derived from Sprague–Dawley (Harlan, Indianapolis, IN) rats bred in our AAALAC-accredited facility. All dams were habituated to the colony room for 2 weeks upon arrival. Prior to mating, dams were handled for 5 min daily for 5 days, with 3 cc/kg of 0.9% saline administered via subcutaneous injection daily during the final 3 days of handling. Following this handling and injection procedure, each dam was placed individually in a hanging cage with an adult male at 1600 h daily, and removed the following morning at 0900 h. On detection of a copulatory plug, defined as day 1 of gestation (E1), each dam was individually housed in an opaque Plexiglas breeding cage.

Prenatal and Postnatal Treatments

Dams were randomly assigned to either cocaine (C40), pair-fed (PF4), or nontreated (NT) treatment groups, and given food (powdered Purina lab chow) and water ad lib throughout the study, unless otherwise noted. C40 dams were subcutaneously injected with 40 mg/kg/3 cc cocaine hydrochloride daily between 1000 and 1200 h from E8–E20. Injections were made in a volume of 3 cc/kg to minimize cocaine-induced skin necrosis, and injections sites varied daily [see (54) for further discussion]. PF4 dams received identical injections of 0.9% saline, with each being pair fed to a cocaine-exposed dam for the first 4 days of cocaine injections (E8–E11). Because cocaine-induced anorexia is only evident for 3–4 days following onset of injections (55), this restricted pair-feeding procedure was chosen to mimic the transient anorexic effects of cocaine administration while minimizing the amount of time that the dams were exposed to this stressful food restriction [see (56), for discussion]. NT dams were not injected. Daily body weights and intake of food and water were recorded from E1 to parturition, with the day of birth designated postnatal day 0 (P0).

On P1, all pups in each litter were weighed, and the total number of pups of each sex recorded. Each litter was culled to 10 pups whenever possible, and fostered to an untreated surrogate dam that gave birth to a litter within the preceding 24–72 h [see (18,20) for further discussion on fostering]; litters containing less than six surviving offspring were not used in these experiments.

Subjects. Ninety-five male and female offspring were examined, with seven to nine offspring placed into each of the 12 test conditions defined by the 3 (prenatal treatment) $\times$ 2 (handling) $\times$ 2 (gender) factorial design. No more than one male and one female from each litter was assigned to this experiment, with the remaining offspring being assigned to other research projects. Offspring were weaned at 21 days of age, and housed in same-sex pairs in wire hanging cages. Unless otherwise stated, animals were given ad lib access to food and water in a temperature- and humidity-controlled vivarium maintained on a 14/10 on/off light–dark cycle, with the lights on at 0700 h. The rats were handled and maintained in accordance with the guidelines of the National Institutes of Health regarding the principles of animal care.

Early handling. Half of the experimental litters from each prenatal treatment condition were subjected to an early "handling" and separation procedure generally modeled after that used by Meaney and colleagues [e.g., (49)], and used previously in our laboratory (8) and by various other groups [e.g., (12,42)]. This early handling/separation procedure consisted of removal of the surrogate dam from the home nest and placement of the pups individually into Plexiglas compartments (6 $\times$ 6 cm) for 15 min daily from P2–P12. Following this daily handling procedure, pups were returned to the home nest and reunited with their surrogate dam. Nonhandled litters were left undisturbed except for usual biweekly cage cleaning.

SIP Testing Procedure

As young adults (P59), animals were singly housed in wire hanging cages. Beginning the following day (P60), animals were weighed and handled for 5 min daily until completion of testing. Following the initial 3 days of isolation housing and handling (P63), each animal was food restricted and gradually reduced to 80–85% of its estimated adult (P90) free-feeding body weight. These estimates of adult (P90) free-feeding body weights were based on pilot data, and were calculated as 15% greater than P60 free-feeding weights for males and 10% greater for females. Once animals reached and maintained their adult food-restricted weight for 4 days, the SIP testing procedure was initiated.

All test sessions were conducted between 1100 and 1300 h by an experimenter blind to each subjects' prenatal treatment and handling conditions. Animals were given 18 daily tests in standard sound-attenuated operant chambers (Med-Associates, 24 $\times$ 30.5 $\times$ 29 cm), with a recessed food trough centered along one wall and a metal water spout attached to a graduated glass water bottle located 8 cm to the right of this food trough. A chamber light located above the food trough was illuminated during all testing procedures. Day 1 of testing consisted of placing the rat into the operant chamber for 30 min, with 30 food pellets (Noyes, 45 mg) in the food trough and free access to water. Water intake recorded on this day served as a measure of baseline water intake. For all subsequent days of testing, one 45-mg food pellet was dispensed into the food trough every 60 s (FT60) for 30 min and water intake (in ml) during the test session was recorded as a measure of SIP.

Following 13 days of standard SIP testing, animals received four days of drug challenges, with animals injected intraperitoneally on each day and returned to their home cages for 10 min before being placed into the operant chambers for the day's SIP session. On days 14 and 16 of SIP, all animals were injected with 0.9% saline in a volume of 2 ml/kg. On SIP days 15 and 17, animals received 5 or 10 mg/kg/2 cc cocaine HCl, with the order of these injections counterbalanced across animals within treatment groups.

Statistical Analyses

Data were analyzed by analysis of variance (ANOVAs). All ANOVAs were tested for nonhomogeneity of variance or violations of sphericity (Hartley F-Max and Maucheley's sphericity test, respectively). In instances where these assumptions were violated, square root transformations of the data, along with Greenhouse/Geisser and Huynh/Feldt adjustments, were used for analysis. All significant main effects and interactions in the ANOVAs are presented, and were further investigated by planned comparisons, LSD and Tukey's HSD post hoc tests, with probabilities of $p \leq 0.05$ being considered significant in all instances.

RESULTS

Maternal Litter Data

The ANOVAs of maternal percent weight gain during pregnancy and average daily food intake revealed significant main effects of treatment, $F(2, 172) = 11.04$, $p \leq 0.001$, $F(2, 172) = 3.06$, $p \leq 0.001$, respectively, while no significant treatment effects were noted with the water intake data. C40 dams ate significantly less food on average during pregnancy than LC dams, and gained significantly less weight during gestation when compared to both PF4 and LC dams (see Table 1). The ANOVA of offspring body weights on P1 revealed only a significant main effect of gender, $F(1, 149) = 118.94$, $p \leq 0.001$, with male pups weighing more than female pups (see Table 1).

The ANOVA of maternal weights across days of pregnancy revealed a significant main effect of prenatal treatment, $F(2, 172) = 12.02$, $p \leq 0.001$, and day, $F(19, 3268) = 1617.81$, $p \leq 0.001$, and a significant interaction involving these two variables, $F(38, 3268) = 8.57$, $p \leq 0.005$ (see Fig. 1a). C40 animals generally weighed significantly less than both NT and PF4 animals, an effect that appears to be partly a result of a sampling bias, as it was present from the first day of pregnancy. Differences among the treatment groups generally became more pronounced following the onset of the cocaine exposure period on embryonic day 8 (E8), with NT dams weighing significantly more than C40 and PF4 dams from E8 through E12, with these latter two groups not differing from each other at the time. Following the 4 days of pair feeding. C40 dams weighed significantly less than PF4 and NT dams from E13 through parturition, while differences between PF4 and NT dams were evident from E16 on. The ANOVA of maternal food intake during pregnancy revealed a significant main effect of prenatal treatment, $F(2, 172) = 3.06$, $p \leq 0.05$ and day, $F(19, 3268) = 49.78$, $p \leq 0.05$, with a significant interaction involving these two variables, $F(38, 3268) = 6.83$, $p \leq 0.001$ (see Fig. 1b). Tukey's post hoc analyses revealed significant differences in food intake on days 8 through 11 (i.e., the first 4 days of injections and pair feeding), with C40 dams and their PF4 counterparts consuming significantly less food than NT dams. A rebound effect was observed on the first day following resumption of free feeding in the PF4 group, with PF4 dams consuming significantly more food than C40 dams on E12. The ANOVA of maternal water intake during pregnancy revealed a significant main effect of day, $F(19, 3268) = 286.24$, $p \leq 0.001$, tempered by a significant interaction of prenatal treatment × day, $F(38, 3268) = 5.27$, $p \leq 0.001$ (see Fig. 1c). Tukey tests revealed that C40 and PF4 dams consumed significantly less water than NT dams on gestational days 8 through 10.

Pretest Body Weights, Days to Reach Deprivation Weight, and Baseline Water Intake

A 3 (prenatal treatment) × 2 (handling) × 2 (gender) ANOVA of P60 body weights revealed a significant main effect of prenatal treatment, $F(2, 82) = 7.74$, $p \leq 0.001$, with a significant interaction involving prenatal treatment × handling, $F(2, 82) = 3.37$, $p \leq 0.05$. Post hoc analyses of these body weights taken 24 h after the onset of single housing revealed that nonhandled C40 animals (mean ± SEM: 223.9 ± 11.9 g) weighed significantly less than nonhandled NT (258.1 ± 13.4) and PF4 (252.6 ± 14.4) offspring. Post hoc analyses revealed no significant differences among the prenatal treatment conditions in handled animals. There was also a main effect of gender, $F(1, 82) = 359.12$, $p \leq 0.001$, with males (290.7 ± 4.6) weighing more than females (196.7 ± 2.4). Correlations conducted within each gender comparing SIP performance with P60 body weights as well as daily body weights while performing the SIP task revealed no significant correlations between body weight and the development or maintenance of SIP across days.

A 3 (prenatal treatment) × 2 (handling) × 2 (gender) ANOVA of the number of days to reach appropriate deprivation weight revealed a significant main effect of prenatal treatment, $F(2, 68) = 3.20$, $p \leq 0.05$, with no significant main effects or interactions involving handling or gender. Animals in the PF4 prenatal condition took significantly longer to reach their appropriate deprivation weight (25.5 ± 1.6) days than both C40 (20.2 ± 1.2) and NT (20.7 ± 1.7) offspring.

The ANOVA of baseline water intake in response to the massed pellet presentation on the first day in the operant chambers revealed no significant main effects or interactions of prenatal treatment, handling, or gender.

SIP Data

The test for sphericity conducted on the SIP water intake data (ml consumption/day) revealed a violation of this statistical assumption and, hence, square root transformations were ap-

TABLE 1

MATERNAL AND OFFSPRING SUMMARY DATA DERIVED FROM LITTER MEANS (±SE) FOR THE THREE EXPERIMENTAL GROUPS

Prenatal Treatment	NT (Mean ± SE)	C40 (Mean ± SE)	PF4 (Mean ± SE)
Percent gestational weight gain	41.26 ± 0.94	35.00 ± 0.88*	38.36 ± 0.92
Average food intake	26.16 ± 0.34	24.90 ± 0.44†	25.22 ± 0.32
Average water intake	61.60 ± 1.07	59.86 ± 0.84	59.10 ± 0.73
Number of pups/litter			
Male	6.39 ± 0.31	5.71 ± 0.27	5.89 ± 0.32
Female	5.85 ± 0.30	6.48 ± 0.33	5.47 ± 0.30
Offspring body weights			
Male	6.70 ± 0.07	6.59 ± 0.07	6.67 ± 0.20
Female	6.46 ± 0.08	6.29 ± 0.07	6.28 ± 0.19

NT = nontreated control group; PF4 = pair-fed control group; C40 = cocaine-treated group.

*Significantly different from LC and PF4 dams.

†Significantly different from LC dams.

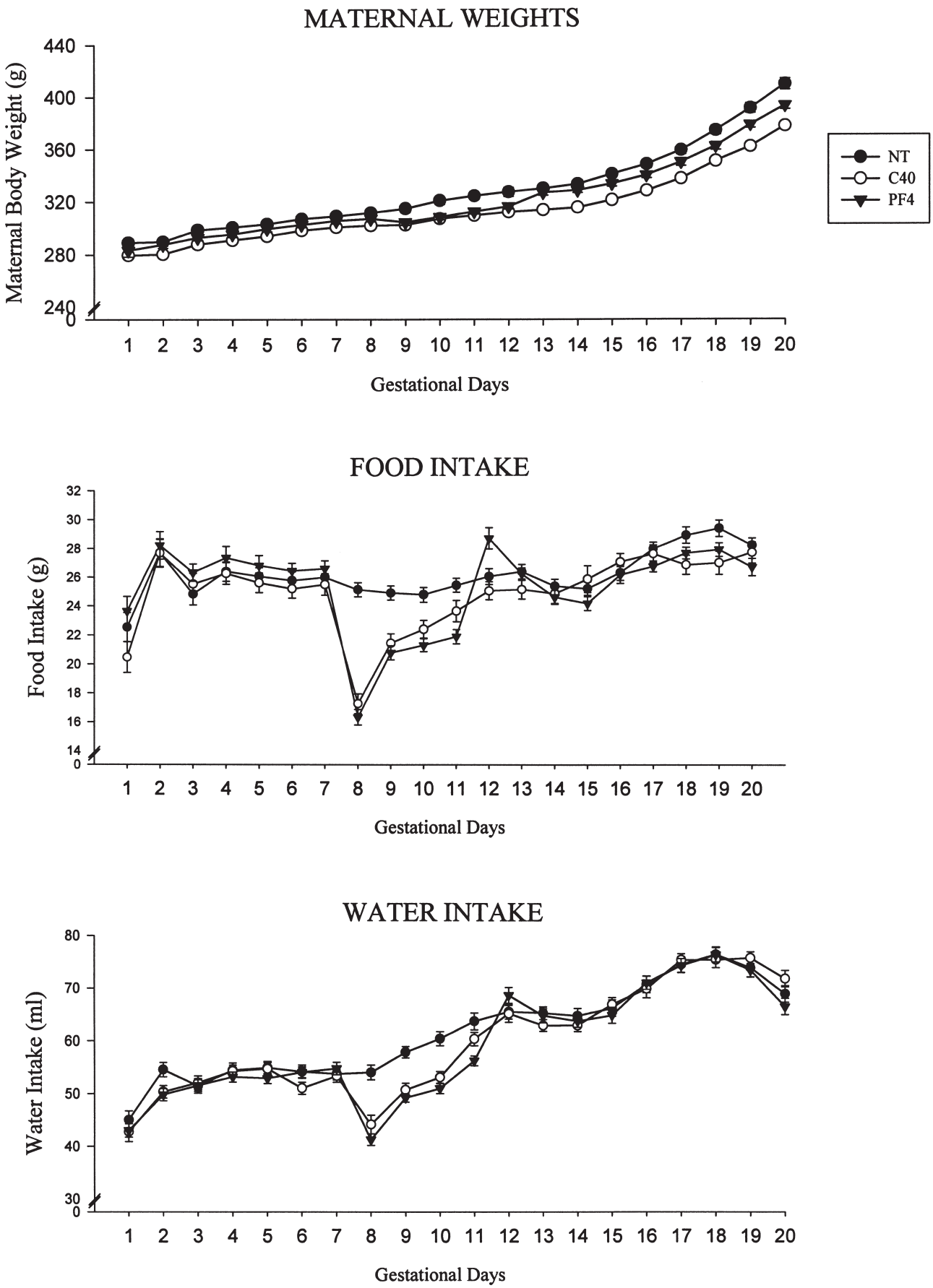

FIG. 1. Mean amount (±SE) of (a) weight gain, (b) food intake, and (c) water intake during gestation of dams from each of the prenatal treatment groups. (NT = nontreated; PF4 = pair fed; C40 = cocaine).

plied to these data before they were analyzed via a 3 (prenatal treatment) × 2 (handling) × 2 (gender) × 13 (day) repeated measure ANOVA. The ANOVA revealed significant main effects of gender, $F(1, 83) = 31.52$, $p \leq 0.001$, and days, $F(12, 996) = 130.98$, $p \leq 0.001$, along with a significant interaction involving these two variables, $F(12, 996) = 2.21$, $p \leq 0.01$ (see Fig. 2). Post hoc analyses of the data collapsed across the prenatal treatment and handling conditions revealed that females displayed an initially accelerated acquisition of SIP compared to males, and consumed significantly more water than males across all days of testing. There was also a significant interaction involving prenatal treatment × handling × days, $F(24, 996) = 2.18$, $p \leq 0.005$. As can be seen in Fig. 3a, handling increased the overall level of polydipsia reached by NT animals, with handled NT animals consuming more water than nonhandled NT animals on days 6 through 13 of testing. In contrast, handling accelerated the acquisition of SIP for PF4 and C40 offspring, with both PF- and C40-handled animals drinking significantly more water than their nonhandled counterparts on days 4 and 5 of testing, but not during later sessions. These handling effects eliminated prenatal treatment effects that were evident in nonhandled animals, with nonhandled NT animals generally maintaining lower levels of SIP than nonhandled C40 and PF4 animals, drinking significantly less water than C40 offspring on days 7, 9, 10, 12, and 13, and than PF4 offspring on days 7–13 (see Fig. 3b).

Drug Challenge Data

A 3 (prenatal treatment) × 2 (handling) × 2 (gender) × 2 (day) repeated-measures ANOVA comparing the SIP data on day 13 vs. the first day of saline injections (day 14) revealed a significant main effect of gender, $F(1, 64) = 5.46$, $p \leq 0.05$, and day, $F(1, 64) = 4.76$, $p \leq 0.05$. Females consumed significantly more water (15.8 ± 0.46 ml) than males (14.1 ± 0.71), and day 14 water intake (15.5 ± 0.45) was significantly greater than day 13 water intake (14.8 ± 0.38). No significant interactions were revealed. A similar analysis conducted comparing the two saline injection days (14 and 16) revealed no significant main effects or interactions involving day. Therefore, each animal's average intake of water across these 2 saline days (14 and 16) was used for subsequent dose comparisons. A 3 (prenatal treatment) × 2 (handling) × 2 (gender) × 3 (dose: 0, 5, 10 mg/kg cocaine HCl) × 2 (order: ordering of the cocaine injections) ANOVA conducted on the dose data revealed significant main effects of gender, $F(1, 49) = 6.02$, $p \leq 0.05$, and dose, $F(2, 49) = 121.81$, $p \leq 0.001$, tempered by significant interactions of handling × gender, $F(1, 49) = 7.74$, $p \leq 0.001$, and gender × order × dose, $F(2, 98) = 3.64$, $p \leq 0.05$. Generally, significant dose-related decreases in SIP were seen in animals of both genders, with handled males displaying significantly more SIP when compared with nonhandled males, an effect not seen in females (data not shown).

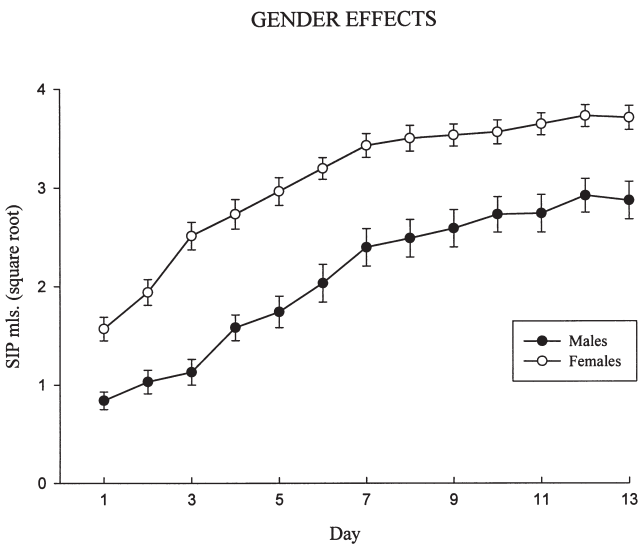

FIG. 2. Square root of the amount of water (in ml) ingested by males and females during each SIP session. Data are collapsed across prenatal treatment and postnatal handling.

DISCUSSION

All three of the independent variables (gender, prenatal treatment, and postnatal handling) elicited significant effects on SIP behavior, although the observed effects were not always in the direction anticipated. As expected, females displayed higher levels of SIP than males throughout testing. Early handling was not observed to decrease SIP as predicted, but rather to increase established levels of SIP in nontreated animals, while accelerating the rate of acquisition of SIP in both pair-fed and cocaine exposed offspring. When examining prenatal treatment effects among nonhandled animals, offspring in the nontreated control group were observed to display significantly less polydipsia following the acquisition of SIP than pair-fed and cocaine-exposed offspring; these effects were opposite from those predicted, and were not evident in handled animals. As previously reported (23), acute challenge with cocaine suppressed SIP, although this pharmacological treatment was not found to reveal consistent effects of gender, prenatal treatment, or early handling.

As predicted, females displayed higher levels of SIP as well as an increased rate of acquisition of polydipsia. Although females typically display higher daily levels of mg/kg fluid intake than males under conditions of ad lib access to food and water in the home cage (32), no significant sex difference among the food-deprived animals in the present study was evident in terms of baseline water intake in the test situation. It was only when the scheduled food delivery was imposed that sex differences in drinking in this test situation emerged, with females averaging 40% greater ml/kg intake by the last day of SIP testing than males, an effect considerably magnified over the typical approximately 15% sex difference in daily intake reported in the home cage (32). Given that animals with high plasma levels of corticosterone typically display high levels of SIP (5), these sex differences in SIP concur with previous research showing that females display a heightened hormonal response to stress (1,27,57). Relating these gender differences in SIP with previous reports of gender differences in mesolimbic DA functioning is less straightforward. Previous research has suggested that either increases or decreases in DA transmission can reduce levels of SIP (36,38), with female rats exhibiting significantly lower basal levels of mesolimbic DA than their male counterparts (5).

Early handling was not observed to decrease SIP performance, an unexpected finding given that animals with low plasma corticosterone levels have been reported to display low levels of SIP (39). Indeed, the early handling procedure used in this experiment was effective in producing the often reported attenuation in the corticosterone response to stress [e,g., (33)], with littermates of these animals exposed to the early handling manipulation exhibiting attenuated restraint-

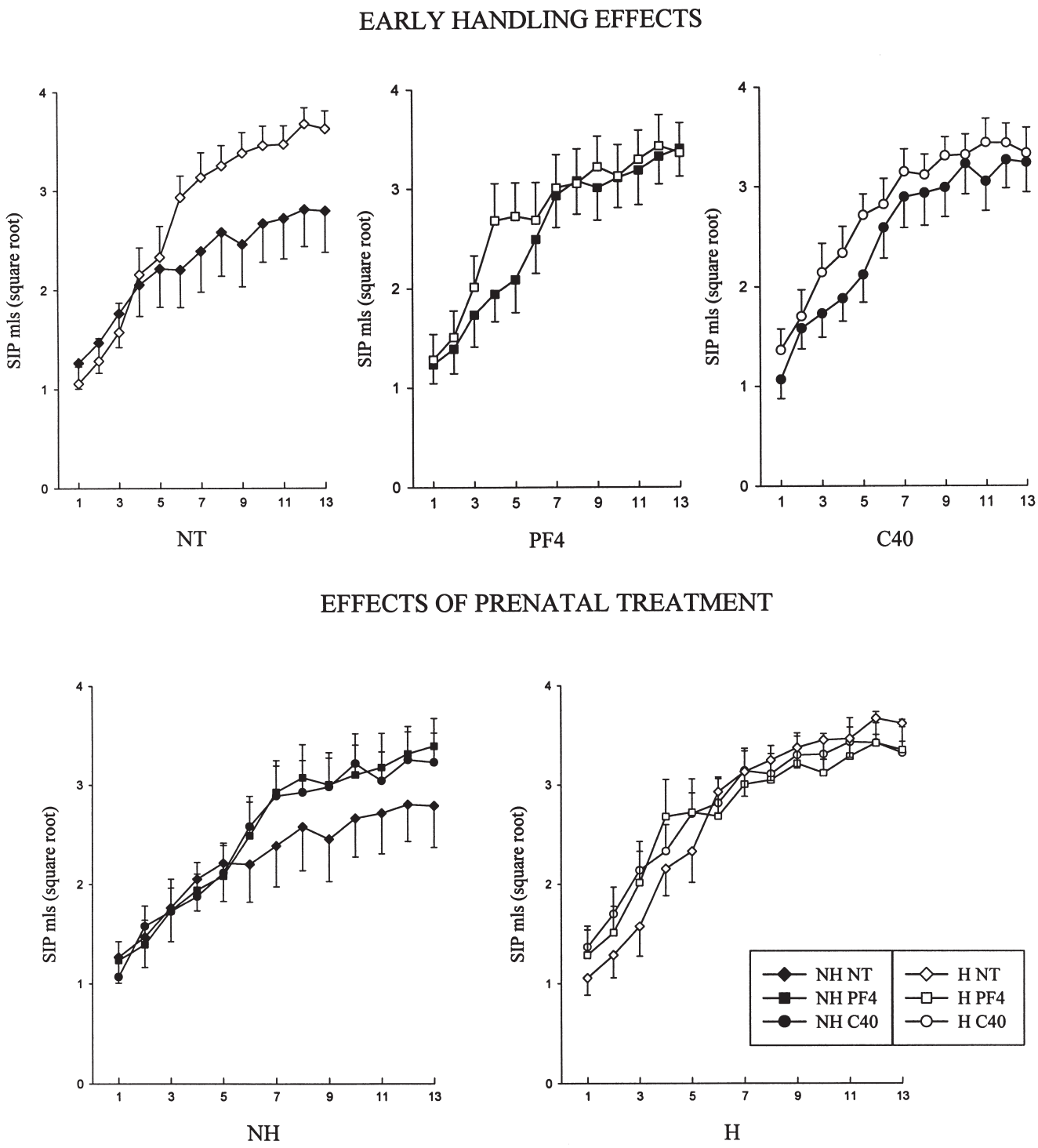

FIG. 3. Square root of the amount of water ingested (in mls) during each SIP session. Data are collapsed across gender and graphed in (a) to contrast the two handling conditions within each of the three prenatal treatment conditions, and in (b) to compare the three prenatal treatment conditions within each of the two handling conditions. (NH = nonhandled; H = handled; NT = nontreated; PF4 = pair-fed; C40 = cocaine).

induced increases in plasma corticosterone relative to those from nonhandled litters (7). Although the observed handling associated increases in SIP acquisition seen in C40 and PF4 offspring and in maintenance of SIP in NT offspring are inconsistent with predictions based on the corticosterone data, there is an alternative interpretation. To the extent that SIP is a coping response (5), and that early handling increases later adaptability to stressors (46), the increased SIP in handled animals could potentially reflect their greater success at coping with the stress elicited by the testing situation.

Viewing SIP as an adaptive coping response presents problems, however, for interpretation of the prenatal treatment data, given that in nonhandled animals it was the C40 and PF4 offspring that displayed the most SIP, with C40 and PF4 offspring exhibiting significantly more SIP that their NT control counterparts following the acquisition of this behavior. Before considering the possible factors that may have contributed to these prenatal treatment effects, it should be noted that the novel pair-feeding approach used in this study was largely unsuccessful for equating nutritional status between the C40 and PF4 dams. Given that pair feeding is a considerable stressor [e.g., (56)], and that prenatal stress influences later stress responsivity [see (53) for discussion and references], a strategy of minimizing the time interval for pair feeding was used in this study. Although this transient pair feeding was successful in matching weight gain and food and water intake between the PF4 and C40 dams during the acute anorexic phase at the onset of drug treatment, following the restoration of free feeding the PF4 group displayed a significant overshoot of food intake. Yet, despite the fact that PF4 dams did not differ significantly in overall pregnancy weight gain from NT dams, the behavior of the PF4 offspring more closely resembled C40 offspring than the offspring of NT dams. It is not clear whether stress or the nutritional consequences of the transient food restriction in PF4 dams contributed to the effects observed in their offspring, nor consequently, whether alterations in SIP performance in C40 offspring are a function of the drug exposure per se or nutritional consequences of that exposure.

In animals reared without the extra stimulation provided by early handling, C40 animals and their PF4 counterparts displayed more SIP than NT animals, an unexpected finding given that in general compromised mesolimbic DA function, such as that seen in C40 offspring (35), would be expected to attenuate SIP behavior. To the extent that there is an inverted U-shaped relationship between DA functioning and SIP performance [e.g., (50–52)], however, it is possible that the attenuation in DA functioning of C40 offspring (25) could have optimized their DA levels for SIP behavior along this inverted U-shaped function, with the net result of increasing levels of SIP maintenance behavior. The alternations in SIP performance seen in C40 offspring and their PF4 counterparts could also potentially be related to prenatal treatment effects on corticosterone, although the report of a sustained increase in plasma corticosterone levels following immobilization stress (10) needs to be balanced against other work showing attenuated HPA reactivity in these offspring (21,30).

When further investigating the impact of developmental manipulations such as prenatal cocaine exposure and postnatal handling on the development and maintenance of SIP, it may prove valuable to investigate neurohormonal function before, during, and after individual SIP sessions and across the acquisition process. Not only does corticosterone differentially influence SIP depending on whether the behavior is being acquired or has been established (11,29), but mesolimbic DA functioning also impacts SIP development and maintenance in a similar manner (45). Moreover, there is a bidirectional influence between an animal's endogenous levels of corticosterone and DA and SIP performance, with the process of SIP testing itself lowering corticosterone levels (5). Although SIP, functioning as a form of adjunctive behavior, has been viewed as a coping response to repeated stress [e.g., (5)], the ramifications of this behavior as an adaptive response for the long-term ability of an animal to exist and thrive remain to be determined.

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