

Prenatal and early postnatal dietary sodium restriction sensitizes the adult rat to amphetamines

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McBride, Shawna M., Bruce Culver, and Francis W. Flynn. Prenatal and early postnatal dietary sodium restriction sensitizes the adult rat to amphetamines. *Am J Physiol Regul Integr Comp Physiol* 291: R1192–R1199, 2006. First published May 4, 2006; doi:10.1152/ajpregu.00774.2005.—Acute sodium deficiency sensitizes adult rats to psychomotor effects of amphetamine. This study determined whether prenatal and early life manipulation of dietary sodium sensitized adult offspring to psychomotor effects of amphetamine (1 or 3 mg/kg ip) in two strains of rats. Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) dams were fed chow containing low NaCl (0.12%; LN), normal NaCl (1%; NN), or high NaCl (4%; HN) throughout breeding, gestation, and lactation. Male offspring were maintained on the test diet for an additional 3 wk postweaning and then fed standard chow thereafter until testing began. Overall, blood pressure (BP), total fluid intake, salt preference, and adrenal gland weight were greater in SHR than in WKY. WKY LN offspring had greater water intake and adrenal gland weight than did WKY NN and HN offspring, whereas WKY HN offspring had increased BP, salt intake, and salt preference compared with other WKY offspring. SHR HN offspring also had increased BP compared with other SHR offspring; all other measures were similar for SHR offspring. The low-dose amphetamine increased locomotor and stereotypical behavior compared with baseline and saline injection in both WKY and SHR offspring. Dietary sodium history affected the rats' psychomotor response to the higher dose of amphetamine. Injections of 3 mg/kg amphetamine in both strains produced significantly more behavioral activity in the LN offspring than in NN and HN offspring. These results show that early life experience with low-sodium diets produce long-term changes in adult rats' behavioral responses to amphetamine.

salt appetite; psychomotor stimulant; behavioral activity

REPEATED EXPOSURES TO HOMEOSTATIC challenges caused by sodium depletion and psychostimulant drugs, such as amphetamines, lead to increased and long-lasting responsiveness to the substance or sensitization (6, 19, 41, 46, 49). Sensitization due to repeated sodium depletion caused by acute injections of furosemide is characterized by increased need-free NaCl intake (19, 46), whereas sensitization to amphetamines is characterized by progressive increases in locomotion, rearing, and stereotypy to repeated amphetamine treatment (20, 41, 49, 55).

Cross-sensitization occurs between sodium deficiency and amphetamine treatment, in which one treatment enhances the response to the other (6). That is, adult rats with prior experience with sodium deficiency show potentiation of the behavioral effects of amphetamines, and rats previously exposed to amphetamines show increased intake of NaCl compared with controls (6). This cross-sensitization between sodium deficiency and amphetamines suggests that a common neural substrate is

affected by the two treatments. One system that has been implicated in both salt and amphetamine sensitization is the mesolimbic dopamine system, which is involved in mediating motivation and reward responses (31, 37, 49, 56). Similar changes in dendritic morphology in the nucleus accumbens (NAc), indicated by increased dendritic branching, length, and number of spines, have been observed in sodium-depleted rats (44) and rats treated with repeated amphetamine injections (43).

In addition to amphetamine and salt sensitization occurring in adult animals, evidence suggests that prenatal and early postnatal experience with these substances can induce sensitization in offspring. For example, prenatal exposure to psychomotor stimulants produces long-term neurochemical and behavioral alterations in offspring, including increased responsiveness to amphetamines (1, 22). Similarly, low-sodium diets during pregnancy produce long-lasting changes in adult offspring, including greater water intake by the offspring and increased adrenal gland weight (3, 9, 10). High-salt intake by the dam during pregnancy also results in permanent physiological and behavioral alterations in offspring, including increased salt intake, salt preference, and blood pressure (BP) (3, 7, 9).

Both dietary sodium manipulation and exposure to psychomotor stimulants during pregnancy have long-lasting effects on offspring. Because cross-sensitization occurs between sodium-depleted rats and amphetamine-treated adult rats, we tested the hypothesis that prenatal and early postnatal dietary sodium restriction would have similar effects to that of sodium restriction in adult rats and sensitize the offspring to amphetamines. Natural challenges, such as sodium deficiency in adults or dietary sodium restriction during early development, act as strong homeostatic challenges, and amphetamines are thought to exploit some of the same neural pathways involved in mediating responses to natural stimuli. Therefore, early life experiences with sodium restriction may alter the brain in a manner that sensitizes the offspring to drugs later in life (6).

In addition, we compared the effects of sodium dietary history and amphetamine cross-sensitization in Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rats because the psychomotor stimulant effects of amphetamine most likely involve dopaminergic function (24), and dopamine neurotransmission is altered in SHR rats (26, 38, 50, 51).

MATERIALS AND METHODS

Subjects

This study was approved by the University of Wyoming Animal Care and Use Committee in accordance with federal regulations.

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WKY and SHR adult male and female rats were obtained from Charles River Laboratories and housed in clear plastic cages in a temperature-controlled room with a 12:12-h light-dark cycle throughout the entire experiment with lights on at 0700. Females were fed a low-NaCl (0.12%; LN), normal-NaCl (1%; NN), or high-NaCl (4%; HN) powdered rat chow diet during breeding, gestation, and lactation, as established in the literature. All diets used in this study allow for normal reproduction, growth, and litter size. The 0.12% NaCl diet was chosen as the LN diet, because this amount is close to the minimum amount required for successful reproduction (0.08% NaCl), and amounts below this lead to high levels of mortality (Refs. 3, 10; B. W. Culver, personal communication). The 1% NaCl diet was chosen as the NN diet because it is similar to standard rat chow. The 4% NaCl diet was chosen as the HN diet, because this concentration is not normally preferred and is sufficiently high to elevate BP (3, 10). Additionally, the various NaCl levels used in this experiment mimic salt intake levels in humans. The 0.12% NaCl diet corresponds to reduced salt diets recommended for people with hypertension, the 1% NaCl diet corresponds to the recommended daily average for salt intake in the United States, and the 4% NaCl diet reflects a HN diet that corresponds with elevated daily salt intake (42).

To produce diets with varied salt content, NaCl was added to sodium-deficient powdered rat chow (MP Biomedicals, Aurora, OH) by weight and mixed for 10 min to ensure homogeneity. Mixed diets were sealed in plastic containers and stored in a cold room. During breeding, female rats were given ad libitum access to the test diet and water during the day. Food was removed in the evening when adult male rats were introduced into the cage and returned in the morning to facilitate breeding. Males were placed in the cages with the females at the end of the day, because mating is most likely to occur during the dark period/night when rats are most active (3). During gestation and lactation, dams were given free access to the test diet and water until pups were weaned. Shortly after birth, litters were adjusted to eight pups per dam, retaining as many male pups as possible, because males were used in the experiment. Litters were culled to an equal number to reduce differences in the postnatal environment (16).

After weaning at postnatal *day 21*, male pups were housed with their littermates and maintained on the test diet for an additional 3 wk with ad libitum access to water. At the end of this period, offspring were switched to standard Purina rat chow pellets and remained on this diet for the duration of the study with free access to both food and water. Prenatal and early postnatal exposure to dietary sodium produces persistent changes in the adult offspring. The time period of sodium manipulation from pregestation to postlactation was chosen because we wanted to maximize the exposure and likelihood of producing an enduring change in the offspring (9). Only male offspring were tested, and testing occurred well into adulthood at 6–7 mo of age. The data from the male offspring within the same litter were averaged to minimize litter effects, with an *n* of 1 for all offspring from the same dam (Table 1).

Our hypothesis involved testing behavioral activity responses to amphetamine in offspring from sodium-restricted, normal, and high-dietary sodium dams; however, systolic BP, fluid intake, and salt preference data were collected as preliminary data to ensure that there were permanent changes in behavior and physiology of adult offspring.

Systolic BP

Systolic BP was measured indirectly using a semiautomated tail-cuff device (IITC, Woodland Hills, CA) before behavioral testing. Rats were acclimated to the testing chamber and restraining tubes for 20 min to reduce restraint stress. Three BP measurements were then taken for each rat, and the average of the three readings was used for the BP. BP was measured in the morning to midafternoon (between 0900 and 1400).

Table 1. *Test groups*

Treatment	WKY		SHR	
	Dams	Offspring	Dams	Offspring
LN	3	15	4	20
1 mg/kg D-amphetamine	3	8	4	10
3 mg/kg D-amphetamine	3	7	4	9
NN	2	8	3	20
1 mg/kg D-amphetamine	2	4	3	10
3 mg/kg D-amphetamine	2	4	3	10
HN	2	7	2	14
1 mg/kg D-amphetamine	2	4	2	8
3 mg/kg D-amphetamine	2	3	2	6

Data is number of rats in group. All offspring from the same litter/same dam were averaged. Preliminary data were collected first using all offspring to determine whether there were permanent changes in adult physiology and behavior of offspring. Next, responses to D-amphetamine were tested, and rats within a dietary group were randomly divided into low- and high-dose amphetamine groups. WKY, Wistar-Kyoto rats; SHR, spontaneously hypertensive rats; LN, low-sodium diet; NN, normal-sodium diet; HN, high-sodium diet.

Two-Bottle Fluid Intake and Salt Preference Test

Adult offspring from LN, NN, and HN groups were examined for differences in fluid intake and NaCl preference (NaCl intake/total intake \times 100). Rats were moved from group cages into individual suspended steel-mesh cages and kept in a temperature-controlled room with a 12:12-h light-dark cycle with lights on at 0700. During tests, rats had ad libitum access to Purina rat chow. Two calibrated glass bottles, one containing deionized water and one containing 0.3 M NaCl, were attached to the front of each cage and placed on opposite sides to prevent mixing of solutions. After 24 h, intake was measured to the nearest milliliter.

Behavioral Activity Testing

Behavioral activity (locomotion, stereotypy, and rearing) were recorded using an activity monitoring system (Omniscan Instruments, Columbus, OH), similar to that commonly used to characterize the effects of psychomotor stimulants (6, 20, 28, 36, 55). Previous studies examining the effects of nicotine on locomotor activity, stereotypy, and rearing behavior employed these monitors and validated their data collection (36). The Digiscan monitor uses arrays of infrared photo-beams mounted on the sides (vertically at 1–3 in. and 6.5–8.5 in.) of the clear acrylic test chambers (16.5 \times 16.5 \times 12 in.). Locomotion or horizontal movement was detected by beam breaks of lower photocells, and rearing or vertical movement was detected by beam breaks of higher photocells. Stereotypy or the number of repetitive movements was monitored by repeated breaks in the same beam in the horizontal plane (28, 36). These behaviors are associated with the psychomotor effects of amphetamines (20, 55).

Activity tests were conducted during the daytime, between 0900 and 1400. During activity testing, offspring were placed in the activity chamber. Rats were allowed to acclimate to the chamber for 30 min; the last 10 min of the acclimation period was recorded as baseline. After acclimation and baseline measurements, rats were removed from the chamber, given an intraperitoneal injection of isotonic saline, and returned to the chamber for 30 min. After 30 min, rats were again removed, and one-half of the rats were given an intraperitoneal injection of 1 mg/kg amphetamine, and the remaining rats were given 3 mg/kg amphetamine (D-amphetamine sulfate; Sigma, St. Louis, MO). Rats were returned to the activity chamber for 30 min, and their behavior monitored. Two different doses of amphetamine were used to determine whether there were dose-dependent interactions with perinatal sodium dietary history (6, 55). The doses are consistent with

those typically used to show psychomotor effects of amphetamine and cross-sensitization to NaCl in adult rats (6, 55).

Adrenal Gland Weight

After the completion of behavioral testing, rats were killed, and the adrenal glands were removed. Wet weight (nearest 0.01 g) of the adrenal gland was obtained to determine the effects of prenatal and early postnatal dietary sodium manipulation on adrenal gland weight.

Data Analysis

The laboratory rat is a multiparous species, with dams producing multiple offspring. This fecundity can lead to problems in statistical analysis for experiments involving prenatal and early postnatal development and effects on offspring. Pups within a litter are genetically alike and share the same intrauterine environment, and the problem arises that offspring within a litter may be more alike than offspring between litters (25). To avoid false inflation of the sample size and as standard in the literature, we averaged all measurements from offspring from the same litter. Male offspring within a litter were therefore treated as $n = 1$ for statistical analyses (3, 13).

Data were expressed as a mean \pm SE, and data were analyzed using SPSS software with a P value ≤ 0.05 considered significant. BP, fluid intake, salt preference, body weight, and adrenal gland weight were analyzed using two-way ANOVA to compare differences between strain and diet. One-way ANOVA followed by post hoc least significant difference analysis was used when differences occurred to examine variance between dietary groups within individual rat strains.

Behavioral activity measurements were analyzed using a three-way ANOVA, with strain, diet, and drug treatment as factors. Significant main effects and interactions were broken down using one-way ANOVA and post hoc least significant difference analyses.

RESULTS

Systolic BP

The significant main effect of strain [$F(1,10) = 123.8, P < 0.001$] reflected that, overall, SHR offspring had significantly greater BP compared with WKY offspring. The main effect of diet [$F(2,10) = 10.6, P < 0.003$] was further analyzed to show that high-dietary sodium significantly increased BP compared with the NN and LN diet in both strains ($P < 0.05$). As such, the BP of both WKY and SHR offspring was affected similarly by early life exposure to high-dietary sodium. There was no interaction between strain and dietary treatment (Table 2).

Body Weight

Overall, WKY offspring weighed more than SHR offspring [$F(1,10) = 170.3, P < 0.001$]. There was no main effect of diet on body weight or interaction between strain and dietary treatment (Table 2).

Two-Bottle Fluid Intake and Salt Preference Test

Fluid intake and salt preference were expressed as a function of body weight.

Water intake. A diet \times strain analysis revealed main effects of strain [$F(1,10) = 7.4, P < 0.02$] and diet [$F(2,10) = 5.1, P < 0.03$] on water intake. SHR offspring had significantly greater water intake compared with WKY offspring. Water intake was affected by early dietary sodium in WKY offspring [$F(2,6) = 30.0, P < 0.005$], with LN rats showing increased water intake relative to NN and HN rats ($P < 0.009, P < 0.002$). Water intake was similar for all SHR offspring. Additionally, overall, total fluid intake (water + salt) was greater in SHR than WKY offspring [$F(1,10) = 56.5, P < 0.001$; Table 2].

Salt intake. In addition, there were main effects of strain [$F(1,10) = 51.5, P < 0.001$] and diet [$F(2,10) = 6.2, P < 0.018$] on salt intake. Again, SHR offspring showed significantly greater salt intake compared with WKY offspring. When examining diet, WKY HN offspring showed an increased salt intake [$F(2,6) = 10.9, P < 0.02$] relative to NN and LN offspring ($P < 0.05$). Salt intake was similar for all SHR offspring (Table 2).

Salt preference. Main effects of strain [$F(1,10) = 19.9, P < 0.001$] and diet [$F(2,10) = 5.0, P < 0.031$] for salt preference indicated that SHR offspring had a significantly greater salt preference compared with WKY offspring. WKY HN offspring showed an increased salt preference [$F(2,6) = 10.5, P < 0.03$] relative to NN and LN offspring ($P < 0.05$). Salt preference was similar for all SHR offspring, indicating that SHR offspring were not affected by perinatal dietary sodium (Table 2).

Adrenal Gland Weight

Adrenal gland weight was expressed as a function of body weight. Overall, adrenal gland weights of SHR offspring were

Table 2. Effects of sodium manipulation during the perinatal period on physiology and behavior of offspring

Treatment	BP, mmHg	Body Weight, g	Fluid Intake Over 24 h Corrected for Body Weight, ml			NaCl Preference, %	Adrenal Gland Weight Corrected for Body Weight, mg
			dH ₂ O	0.3 NaCl	Total		
WKY rats							
LN	127 \pm 4	492 \pm 29	9 \pm 0.4*	2 \pm 1	12 \pm 1	22 \pm 6	58 \pm 7*
NN	121 \pm 3	572 \pm 32	6 \pm 1	3 \pm 1	9 \pm 1	28 \pm 6	42 \pm 5
HN	144 \pm 4*	549 \pm 16	5 \pm 1	7 \pm 1*	12 \pm 1	56 \pm 7*	37 \pm 1
SHR rats							
LN	171 \pm 5	336 \pm 5	10 \pm 2	17 \pm 3	28 \pm 3	58 \pm 8	74 \pm 3
NN	162 \pm 5	356 \pm 6	10 \pm 2	13 \pm 2	23 \pm 3	54 \pm 2	80 \pm 6
HN	189 \pm 1*	340 \pm 7	11 \pm 3	19 \pm 1	30 \pm 2	64 \pm 8	77 \pm 2
Total WKY	130 \pm 5	531 \pm 20*	7 \pm 1	4 \pm 1	11 \pm 1	31 \pm 7	47 \pm 4
Total SHR	172 \pm 4*	344 \pm 4	10 \pm 1*	16 \pm 2*	26 \pm 2*	58 \pm 4*	77 \pm 2*

Values are means \pm SE, with all offspring from the same litter averaged; $n = 3, 2,$ and 2 for WKY LN, NN, and HN; $n = 4, 3,$ and 2 for SHR LN, NN, and HN (see Table 1). Preliminary data were used to determine whether there were permanent changes to adult offspring. BP, blood pressure; dH₂O, deionized water. *Significantly different from other dietary groups for within-strain comparisons (LN vs. NN/HN or HN vs. NN/LN) or significantly different from other strain for between-strain comparisons (WKY vs. SHR).

greater than those of WKY offspring [$F(1,10) = 86.1, P < 0.001$]. A significant strain \times diet interaction [$F(2,10) = 6.4, P < 0.016$] was further analyzed and showed that only WKY rats displayed a significant effect of diet on adrenal gland weight, which was reflected in the fact that the adrenal gland weights of WKY LN offspring were significantly greater than those of offspring reared on the NN and HN diets ($P < 0.048, P < 0.025$). SHR offspring adrenal gland weights were not significantly different from one another (Table 2).

Behavioral Activity Tests: 1 mg/kg Amphetamine

Locomotion. There was no significant effect of dietary sodium on locomotion with any of the groups' responses to the low dose of amphetamine. There were significant main effects of drug treatment on locomotor behavior [$F(2,30) = 86.4, P < 0.001$], reflecting that the low dose of amphetamine increased locomotion and stereotypy in all offspring compared with baseline and saline injection ($P < 0.001$). The significant strain \times drug treatment interaction for locomotion [$F(2,30) = 7.3, P < 0.003$] revealed that the WKY offspring displayed significantly greater locomotion than SHR offspring after amphetamine administration [$F(1,15) = 6.6, P < 0.023$; Table 3].

Stereotypy. Dietary sodium history did not significantly affect any of the groups' stereotypical responses to the low dose of amphetamine. Similar to locomotor behavior, significant main effects of drug treatment on stereotypy [$F(2,30) = 19.0, P < 0.001$] indicated that the amphetamine treatment increased stereotyped behavior in all offspring compared with baseline and saline measurements ($P < 0.05$). A significant strain \times drug treatment interaction [$F(2,30) = 6.0, P < 0.006$] also revealed that WKY offspring displayed significantly greater stereotyped behavior compared with SHR after amphetamine treatment [$F(1,15) = 6.6, P < 0.023$; Table 3].

Rearing. Rearing, in response to the low dose of amphetamine, was not significantly affected by dietary sodium history for any of the groups. Additionally, there was no main effect of drug treatment on rearing, but there was a significant interaction between drug treatment and strain [$F(2,30) = 4.3, P < 0.022$]. SHR offspring showed significantly more rearing during baseline and after saline injection compared with WKY offspring [$F(1,15) = 16.3, P < 0.001$; $F(1,15) = 5.4, P < 0.04$; Table 3].

Behavioral Activity Tests: 3 mg/kg Amphetamine

Locomotion. Three-way ANOVA revealed main effects of diet on locomotion [$F(2,30) = 10.6, P < 0.001$], showing that the LN offspring had increased locomotion compared with the other groups ($P < 0.05$). The significant diet \times drug treatment interaction for locomotion [$F(4,30) = 8.2, P < 0.001$] was further analyzed and indicated that the WKY offspring showed no differences during baseline or following saline injection. However, locomotion [$F(2) = 5.3, P < 0.02$] was significantly increased in WKY LN offspring compared with NN offspring ($P < 0.05$) after amphetamine treatment. SHR offspring also showed no differences during baseline or after saline injection. However, following injection of 3 mg/kg amphetamine, locomotion [$F(2,18) = 4.9, P < 0.02$] was increased in the SHR LN offspring compared with NN offspring ($P < 0.037$). These results revealed that both WKY and SHR offspring exposed to the LN diet during the perinatal period were sensitized to the psychomotor stimulant effects of amphetamine. Locomotor behavior was comparable between both WKY and SHR NN and HN offspring after amphetamine treatment.

There were also main effects of drug treatment on locomotor behavior [$F(2,30) = 40.7, P < 0.001$]. Similar to the low dose of amphetamine, 3 mg/kg amphetamine increased locomotion compared with baseline and saline measurements ($P < 0.05$). The significant strain \times drug treatment interaction for locomotion [$F(2,30) = 3.9, P < 0.031$] was further analyzed into the simple main effects, with SHR offspring showing increased locomotion compared with WKY offspring during baseline measurements [$F(1,15) = 25.1, P < 0.001$] and after saline injection [$F(1,15) = 12.2, P < 0.004$]. See Table 4 and Fig. 1.

Stereotypy. When evaluating stereotypy, main effects of diet on stereotypy [$F(2,30) = 5.3, P < 0.011$] revealed that the LN offspring had increased behavior compared with the other groups ($P < 0.05$). The significant diet \times drug treatment interaction for stereotypy [$F(2,30) = 6.6, P < 0.001$] was further analyzed and showed that there were no differences between WKY offspring during baseline or following saline injection. Stereotypy was increased [$F(2) = 4.1, P < 0.04$], however, after amphetamine treatment in WKY LN offspring compared with NN offspring ($P < 0.04$). Likewise, SHR offspring also showed no differences during baseline or after

Table 3. Behavioral activity measurements during baseline, after saline, and after 1 mg/kg amphetamine injection

Treatment	Locomotion			Stereotypy			Rearing		
	Baseline	Saline	D-Amphetamine	Baseline	Saline	D-Amphetamine	Baseline	Saline	D-Amphetamine
WKY rats									
LN	1,223 \pm 227	997 \pm 38	4,063 \pm 467	66 \pm 28	59 \pm 13	130 \pm 19	4 \pm 2	4 \pm 2	15 \pm 7
NN	1,022 \pm 389	762 \pm 177	4,869 \pm 271	66 \pm 6	45 \pm 12	222 \pm 83	6 \pm 3	5 \pm 1	19 \pm 4
HN	1,086 \pm 203	1,051 \pm 243	3,782 \pm 568	66 \pm 5	70 \pm 23	162 \pm 27	6 \pm 5	7 \pm 3	12 \pm 0
SHR rats									
LN	1,033 \pm 73	941 \pm 59	2,908 \pm 439	39 \pm 6	47 \pm 2	107 \pm 32	13 \pm 2	7 \pm 2	25 \pm 10
NN	1,347 \pm 226	1,320 \pm 192	2,719 \pm 689	47 \pm 11	50 \pm 11	63 \pm 11	19 \pm 3	21 \pm 4	5 \pm 1
HN	1,229 \pm 8	1,165 \pm 11	4,103 \pm 423	50 \pm 1	44 \pm 4	101 \pm 13	19 \pm 5	11 \pm 3	9 \pm 5
Total WKY	1,126 \pm 150	946 \pm 95	4,499 \pm 433*	66 \pm 11	58 \pm 10	194 \pm 41*	5 \pm 2	5 \pm 1	15 \pm 3
Total SHR	1,181 \pm 87	1,117 \pm 85	3,110 \pm 340	44 \pm 4	47 \pm 3	91 \pm 16	16 \pm 2*	13 \pm 3*	15 \pm 5

Values are means \pm SE, with all offspring from the same litter averaged; $n = 3, 2,$ and 2 for WKY LN, NN, and HN; $n = 4, 3,$ and 2 for SHR LN, NN, and HN (see Table 1). Measurements of locomotion, stereotypy, and rearing show the accumulated counts during baseline (10 min \times 3), after saline injection (30 min), and after 1 mg/kg amphetamine injection (30 min). *Significantly different from other dietary groups for within-strain comparisons (LN vs. NN/HN or HN vs. NN/LN) or significantly different from other strain for between-strain comparisons (WKY vs. SHR).

Table 4. Behavioral activity measurements during baseline, after saline, and after 3 mg/kg amphetamine injection

Treatment	Locomotion			Stereotypy			Rearing		
	Baseline	Saline	D-Amphetamine	Baseline	Saline	D-Amphetamine	Baseline	Saline	D-Amphetamine
WKY rats									
LN	725±95	955±138	6,321±1,162*	35±14	43±2	306±30*	2±1	7±4	14±2
NN	892±165	722±1	3,308±222	54±5	35±6	237±33	9±5	5±3	6±4
HN	390±178	576±60	2,372±878	27±11	56±20	118±46	2±2	1±1	1±1
SHR rats									
LN	1,313±151	1,182±162	4,279±1,251*	48±6	39±3	211±39*	20±2	14±3	19±6
NN	1,415±95	1,488±113	2,589±216	56±14	62±15	128±25	18±4	17±4	17±7
HN	1,190±88	1,023±42	2,082±233	58±10	53±12	88±12	17±2	12±1	11±2
Total WKY	677±109	780±85	4,332±876	35±8	44±7	232±38*	4±2	4±2	8±3
Total SHR	1,320±76*	1,249±98*	3,227±618	53±5	50±6	156±25	19±2*	14±2*	16±3

Values are means ± SE, with all offspring from the same litter averaged; $n = 3, 2,$ and 2 for WKY LN, NN, and HN; $n = 4, 3,$ and 2 for SHR LN, NN, and HN (see Table 1). Measurements of locomotion, stereotypy, and rearing show the accumulated counts during baseline (10 min × 3), after saline injection (30 min), and after 3 mg/kg amphetamine injection (30 min). *Significantly different from other dietary groups for within-strain comparisons (LN vs. NN/HN or HN vs. NN/LN) or significantly different from other strain for between-strain comparisons (WKY vs. SHR).

saline injection. However, following injection of 3 mg/kg amphetamine, stereotypy [$F(2,18) = 11.6, P < 0.001$] was increased in the SHR LN offspring compared with NN offspring ($P < 0.05$), again suggesting that offspring exposed to the LN diet during the perinatal period were sensitized to amphetamine. Stereotyped behavior was comparable between both WKY and SHR NN and HN offspring after amphetamine treatment.

The main effects of drug treatment [$F(2,30) = 45.0, P < 0.001$] showed increased stereotypical behavior in all offspring after amphetamine treatment compared with baseline and saline measurements ($P < 0.05$). The significant strain × drug treatment interaction for stereotypy [$F(2,30) = 6.7, P < 0.004$] was further analyzed into the simple main effects, showing that WKY offspring had increased stereotypy compared with SHR offspring after amphetamine injection [$F(1,15) = 5.5, P < 0.04$]. See Table 4 and Fig. 2.

Rearing. There were no main effects of diet on rearing behavior. However, significant main effects of drug treatment

on rearing [$F(2,30) = 4.5, P < 0.02$] showed that rearing, unlike locomotion or stereotypy, was higher during the baseline period than after saline or amphetamine injection ($P < 0.05$). A significant strain × drug treatment interaction for rearing [$F(2,30) = 3.4, P < 0.048$] was further analyzed and showed that SHR offspring had greater rearing behavior compared with WKY offspring during baseline [$F(1,15) = 38.7, P < 0.001$] and after saline treatment [$F(1,15) = 12.3, P < 0.003$]. See Table 4 and Fig. 3.

DISCUSSION

There are several parallels between repeated sodium depletions and repeated amphetamine treatments. Rats exposed to repeated sodium depletion show a behavioral sensitization to subsequent experiences with salt solutions (46). Similarly, multiple experiences with psychomotor stimulants, such as amphetamines, lead to behavioral sensitization, as shown by increased responsiveness to amphetamines (47, 52). Previous studies have established a link between sodium depletion and amphetamine sensitivity. Clark and Bernstein (6) showed that rats with a prior history of sodium depletions displayed an

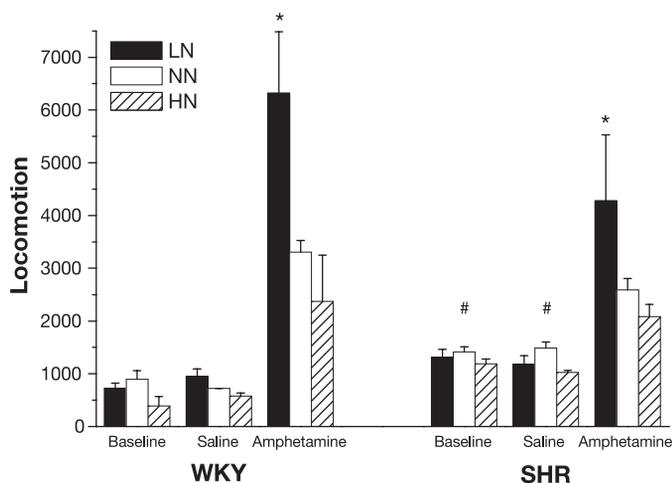


Fig. 1. Mean number of locomotor responses ± SE during 10-min baseline (×3), 30 min after isotonic saline, and 30 min after 3 mg/kg amphetamine injection. WKY, Wistar Kyoto rats; SHR, spontaneously hypertensive rats; LN, low-sodium diet; NN, normal-sodium diet; HN, high-sodium diet. WKY LN: $n = 3$; NN: $n = 2$; HN: $n = 2$; SHR LN: $n = 4$; NN: $n = 3$; HN: $n = 2$ (Table 1). *Significantly different compared with other dietary groups. #Significantly different compared with other strain.

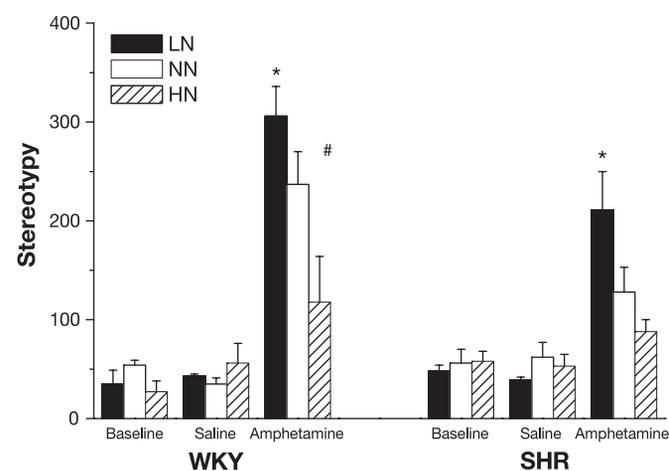


Fig. 2. Mean stereotypical responses ± SE during 10-min baseline (×3), 30 min after isotonic saline, and 30 min after 3 mg/kg amphetamine. No. of rats are as given in Fig. 1 legend. *Significantly different compared with other dietary groups. #Significantly different compared with other strain.

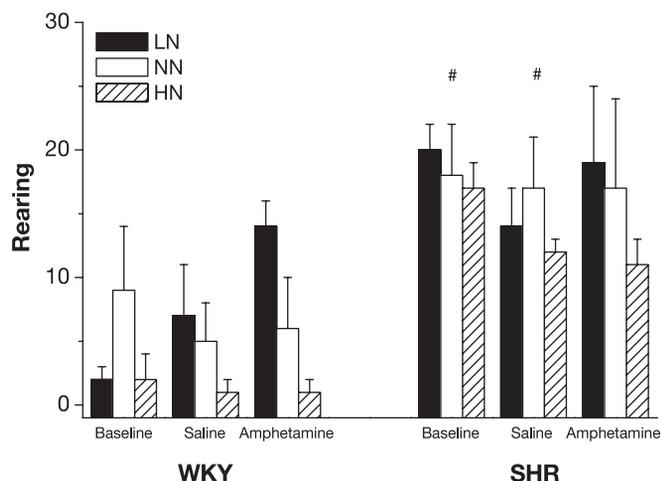


Fig. 3. Mean number of rears \pm SE during 10-min baseline ($\times 3$), 30 min after isotonic saline, and 30 min after 3 mg/kg amphetamine. No. of rats are as given in Fig. 1 legend. #Significantly different compared with other strain.

elevated intake of hypertonic NaCl and were sensitized to the psychomotor stimulant effects of amphetamines. The separate role of sodium depletion and elevated sodium solution intake in the genesis of the cross-sensitization to amphetamines, however, was not specified by this study, and the effects of sodium depletion on brain function and adaptation may be separate from the behavioral manifestation of increased salt appetite.

Prenatal and early postnatal exposure to diets differing in sodium content affects the organization of the brain. Early life experience with sodium affects responsiveness of rats to saline solutions and BP regulation when tested as adults (7, 9). WKY offspring of dams on the LN diet displayed elevated daily water intake compared with offspring of dams on the NN and HN diets, similar to other reports (40), suggesting a reorganization of systems regulating fluid intake (7, 9). In contrast, SHR offspring all drank similar amounts of water. Previous studies show differences between WKY and SHR in thirst responses to water deprivation and hyperosmolarity (27). The mechanisms responsible for different thirst responses to osmotic challenges, and low early dietary sodium levels in SHR offspring are unknown.

The main focus of the current experiment was to determine whether early dietary sodium experience affects the offspring's response to amphetamine when tested as an adult. We tested mature adult rats at 6–7 mo of age and demonstrated a cross-sensitization between low prenatal and early postnatal dietary sodium intake and amphetamine in both SHR and WKY rats. This produced a long-lasting effect on brain function, because rats had been on a normal diet for several months. When examining offspring of dams reared on NN and HN diets, we found that amphetamine had similar psychomotor stimulant effects. In contrast, the offspring of SHR and WKY dams reared on the LN diet were sensitized to the psychomotor stimulant effects of amphetamine. Decreased sodium content in the diet of dams enhanced both the WKY and SHR offspring's psychomotor responses to the higher (3 mg/kg) dose of amphetamine when tested months later as adults, suggesting a threshold response to the drug. These offspring showed greater locomotor behavior and stereotypy after amphetamine administration than did the offspring of dams on the NN diet.

Low prenatal and early postnatal dietary sodium affected locomotion and stereotypy in both WKY and SHR offspring, and thus we find a very similar pattern of behavioral sensitization to amphetamine in the LN SHR and WKY offspring.

Decreased sodium in the diet during the perinatal period appears to have a broader effect on the offspring's psychomotor responses to amphetamine than does acute sodium depletion in naive adults. In previous studies with naive adults, rats given furosemide treatments to arouse a salt appetite displayed more rearing but not horizontal movement (locomotion) in response to amphetamine (6, 44). We observe increases in both locomotion and stereotypy in offspring exposed to the LN diet during the perinatal period. However, the differences in psychomotor responses to amphetamine in offspring reared on LN diets and naive adult rats treated acutely with furosemide may reflect slight differences in the dose of amphetamine, behavioral scoring procedures, and length of exposure to the sodium-deficient stimulus. Regardless, the present results show that early life environmental factors associated with low dietary sodium levels cause a long-lasting sensitization to the psychomotor response to amphetamines in SHR and WKY rats.

Our hypothesis was that maternal dietary sodium manipulations would directly affect offspring, but we also know that these manipulations can affect maternal behavior and mother-pup interactions. For example, studies have shown that dams on HN diets have increased contact with pups during nursing and increased anogenital licking of pups, which aids in water and electrolyte conservation in the dam but may lead to higher BP and salt intake in pups (8). Therefore, in our study, the increased salt intake and BP in offspring from dams maintained on the HN diet during the perinatal period may be related to altered maternal behavior and mother-pup interactions. Perhaps the LN diet during early development also affected maternal behavior and led to altered drug responses in offspring as a result.

Several mechanisms may underlie the early-life programming of amphetamine sensitization by low dietary sodium availability during the perinatal period. Both decreased sodium in the diet and amphetamine treatment may activate a common neurochemical system, specifically the mesocorticolimbic dopamine system (31). A number of studies show that repeated treatment with amphetamines increases dopamine release and activity (31, 56). More recently, studies show that this same system is modified by sodium depletion. Both acute sodium depletion and repeated amphetamine administration promote alteration of dendrites in neurons in the NAc (31, 43, 44). The effects of low maternal dietary sodium on dendritic morphology of NAc neurons have not been examined, but LN diets in early life have been shown to affect neuronal morphology in other brain regions (23, 32, 34, 35).

Another mechanism may involve the hypothalamic-pituitary-adrenal axis. Maternal malnutrition can increase prenatal stress levels (54), and we replicated findings that offspring of dams reared on LN diets had larger adrenal gland weights than offspring of dams on a NN diet (10). Adrenal hypertrophy could reflect effects of stress on corticosterone production and/or effects of plasma sodium on mineralocorticoids. LN offspring also showed the increased psychomotor response to 3 mg/kg amphetamine injections compared with the other dietary groups. Prenatal stress during development affects hypothalamic-pituitary-adrenal axis function and behavior in adult

offspring (29, 53). Offspring of dams that have been subjected to stress show increased locomotor response to the psychomotor stimulant effects of amphetamine (14, 33). The effect of maternal stress on amphetamine sensitivity may involve the dopamine system, which is implicated in sensitization to amphetamines (16, 41, 52). Thus the enhanced behavioral responses to amphetamine in offspring reared on LN diets may involve modulation of dopaminergic neurotransmission within the mesolimbic dopamine system due to dietary-induced maternal stress. While LN dietary-induced stress may contribute to the enhanced psychomotor effects of amphetamine in WKY offspring, the applicability of this explanation for the behavioral sensitization to amphetamine in SHR LN offspring is lessened because SHR offspring all had similar adrenal gland weights. One possible explanation is that the stress response in SHR is not reflected in adrenal gland weight. Additionally, differences in gene expression in the brain have been found between WKY and SHR in response to chronic stress, so adaptations to chronic LN during prenatal and early postnatal periods may not be the same (39).

It has also been suggested that dopaminergic neurotransmission is altered or dysfunctional in SHR, which may account for some of the behavioral changes we observed, including increased basal activity rates and decreased response to amphetamine in SHR compared with WKY (21, 45). Dopamine hypofunction in SHR due to early high levels of dopamine during development could alter neural circuitry in SHR, such that psychostimulants like D-amphetamine act differently in the brain of an SHR vs. a WKY rat (5, 45). Similar to other studies, we also report that SHR had greater basal activity levels than did WKY offspring. Many studies have used the SHR rat as a model of attention-deficit hyperactivity disorder in humans because of their hyperactivity, and, like people with attention-deficit hyperactivity disorder, SHR rats respond to D-amphetamine with decreased activity compared with normal counterparts. Regardless of the possible differences between strains, both the WKY and the SHR offspring from dams exposed to a LN diet during the perinatal period showed sensitization to amphetamine (5, 45).

In addition to dietary-induced differences in water intake mentioned earlier, we show that early-life experience with a HN diet increased BP in SHR and WKY offspring compared with offspring exposed to the LN and NN diets, which is in concordance with other studies (2, 30). Similarly, we show that WKY offspring reared on a HN diet had higher intake of hypertonic NaCl solution than offspring of dams maintained on a NN or LN diet. SHR offspring in all of the dietary groups consumed a high amount of hypertonic NaCl, and there was no further increase in the intake of NaCl as a result of the dietary sodium history. Similar to other reports, the intake and preference for hypertonic NaCl was greater in SHR than in WKY offspring reared on the NN diet (15, 17). Previous studies have examined the effects of early-life exposure to diets of different sodium concentrations on BP in SHR rats (2, 11, 30), but we are not aware of any reports describing the effects of dietary sodium history on the intake of NaCl solutions by the SHR offspring.

In conclusion, this study provides the first evidence that low levels of dietary sodium during pregnancy and early life enhance the sensitivity to amphetamines in adult offspring. The important question is if this LN dietary-induced behavioral

sensitization would predispose or deter the offspring from drug taking. Behavioral sensitization to amphetamine has been associated with increased drug use and drug addiction (4, 18, 52), and our dietary sodium model may similarly predispose the offspring to increased amphetamine use. Ongoing experiments are evaluating this hypothesis. Several possible underlying mechanisms may contribute to the cross-sensitization between early LN diets and amphetamines. Further studies are needed to examine the relationship between LN diets during pregnancy, chronic stress, and alterations of the mesolimbic dopamine systems.

Perspectives

Studies of this nature are of clinical importance because it has been common practice to place women on a LN diet during pregnancy to prevent preeclampsia or gestational hypertension (48). Additionally, dexamethasone, a synthetic glucocorticoid, is sometimes used during pregnancy to prevent preterm labor and congenital adrenal hyperplasia (54). Our study suggests that these treatments may have undesirable effects on the adult offspring. Amphetamine abuse is an increasing problem today, and there is considerable interest in the factors contributing to drug susceptibility in humans. The present results raise the possibility that LN diets during pregnancy and the early postnatal period may be a previously unsuspected contributing factor in individual differences in amphetamine sensitivity and possible drug addiction.

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