Maternal Stress Modulates the Effects of Developmental Lead Exposure

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Lead exposure is higher among children with low socioeconomic status (SES) compared with other children in the United States. Low SES itself is a known risk factor for various diseases and dysfunctions, effects that have been ascribed to chronic stress and associated elevation of glucocorticoids. Chronically elevated glucocorticoids and Pb provoke similar behavioral changes, and both can act on mesocorticolimbic systems of the brain. In this study we examined the hypothesis that these co-occurring risk factors, Pb and environmental stress, would interact and modulate each others' effects. Using a rodent model, we focused on the specific contributions of maternal stress (restraint) and maternal Pb exposure (150 ppm in drinking water) on corticosterone levels of offspring, as well as on neurotransmitter changes and a behavioral baseline (fixed-interval schedule-controlled performance) with known sensitivities to Pb. We observed interactions of Pb and stress that differed in relation to outcome measure and sex. In addition, potentiated effects (effects of Pb plus stress but showing no changes produced by either alone) were observed more frequently in females. Importantly, Pb alone (in males) and Pb plus stress (in females) permanently elevated corticosterone levels in offspring; even short-term Pb exposure to dams could cause this effect. Such increases could suggest a potential new mechanism by which Pb exposure could directly or indirectly enhance susceptibility to diseases and dysfunctions and induce cognitive deficits. Moreover, the interactive effects of Pb and stress, and particularly the potentiated effects of Pb plus stress, raise questions about whether current risk assessment strategies sufficiently consider the potential for modulation of toxicity that can accrue from intercurrent risk factors. *Key words:* **corticosterone, dopamine, frontal cortex, lead, nucleus accumbens, stress, striatum.** *Environ Health Perspect* **112:717–730 (2004). doi:10.1289/ehp.6481 available via** *http://dx.doi.org/* **[Online 29 January 2004]**

Elevated lead exposure is no longer ubiquitous in the United States, but rather has become demographically circumscribed, with exposures now primarily occurring among socioeconomically disadvantaged, medically underserved inner-city minority children who reside in old housing with Pb-based paints. Correspondingly, levels of elevated blood Pb (BPb) in children differ markedly in relation to race/ethnicity, income level, and residence type. For example, the percentage of elevated BPb values in African-American children, at 21.9%, is five times higher than that of the general population; for children from low-income families living in homes built before 1946, typical in large U.S. inner cities, the corresponding figure is 16.4%, almost 4-fold higher than that for the overall population (Pirkle et al. 1998).

Low socioeconomic status (SES) by itself is a significant risk factor for numerous adverse health outcomes, as well as for various behavioral and neurologic dysfunctions, even after controlling for other pertinent covariates. Risk of mortality, prevalence of disease, and increased blood pressure have all been shown to be inversely related to employment grade, occupational status, income, and years of education (Adelstein 1980; Dyer et al. 1976; Marmot et al. 1984; Pappas et al. 1993; Pincus et al. 1987). Low SES has also been associated with an increased prevalence of schizophrenia (Dohrenwend 1990) and depression (Hirschfeld and Cross 1982). In addition to Pb poisoning, links between low SES and adverse health outcomes for children have been reported for vision problems, otitis media, hearing loss, cytomegalic inclusion disease, and iron deficiency anemia (Egbuonu and Starfield 1982). Notably, children in families with lower SES have higher levels of mental retardation, learning disorders, emotional and behavioral problems, and deficits in language, memory, and attentional capacities (Anderson and Armstead 1995; Ardila and Rosselli 1995).

This increased prevalence of adverse health outcomes and behavioral dysfunctions in lower SES populations has been hypothesized to result from the greater environmental stresses experienced by such groups (Lupien et al. 2001). Individuals with lower SES report greater exposures to stressful life events as well as greater impacts of these stresses on their lives (Dohrenwend 1973). Correspondingly, studies show that higher levels of stress are associated with poorer health outcomes, including cardiovascular disease and death, chronic illness, and altered immune function (Brosschot et al. 1994; Calabrese et al. 1987; Kennedy et al. 1988; Rahe and Lind 1971; Wyler et al. 1971). Increased stress has also been shown to adversely affect mood and cognitive function (Lupien and McEwen 1997), and to correlate with measures of anxiety and depression (Vinokur and Selzer 1975). Stress may actually exert its most detrimental and particularly long-lived effects on children (Cohen et al. 1973; Tennes and Kreye 1985).

The relationship between low SES and adverse health effects is thought to be mediated by chronic elevations of stress-associated adrenal cortical glucocorticoids (Lupien et al. 2001), a condition that has been reported to increase resistance to insulin and to cause hypertension, hypercholesterolemia, and arteriosclerosis. Additionally, increased corticosterone is associated with immunosuppression (Munck et al. 1984). The brain is also a target of the adverse effects of prolonged elevation of glucocorticoids (Kerr et al. 1991; Sapolsky et al. 1986), with effects including impairments of cognitive function (Anderson and Armstead 1995; Ardila and Rosselli 1995). The latter effects may ultimately occur through actions on mesocorticolimbic dopamine (DA)/glutamate systems of the brain in prefrontal cortex, nucleus accumbens, and hippocampus (Barrot et al. 2000; Diorio et al. 1993; Kerr et al. 1994; Lowy et al. 1993; McEwen 2001; McEwen et al. 1968; Moghaddam 2002).

Pb exposure likewise targets mesocorticolimbic DA/glutamate systems and thereby adversely affects the associated behavioral processes (Cory-Slechta et al. 1997a, 1997b; Devoto et al. 2001; Lasley et al. 2001; Lasley and Lane 1988; Ma et al. 1998; Nihei et al. 2000; Pokora et al. 1996; Zhang et al. 2002; Zuch et al. 1998). Moreover, Pb has behavioral consequences similar to those reported for stress, including an inverse association with IQ scores in prospective epidemiologic studies (Bellinger et al. 1987; Canfield et al. 2003), deficits in attentional processes (Bellinger et al. 1994; Walkowiak et al. 1998), and higher rates of early high school dropout and juvenile delinquency and criminality (Bradley and Corwyn 2002; Needleman et al. 1996).

In addition to the overlaps in their behavioral and central nervous system effects, Pb

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exposure levels are also highest in lower SES groups, populations that are also considered to experience the highest levels of environmental stress. Two questions arise in the context of these observations: *a*) Do these two risk factors, Pb and environmental stress, interact, and if so, how does this modulate the health risks associated with Pb exposure? Almost nothing is known at present about such a possibility, but some suggestive findings have been reported from human studies. For instance, low SES can modulate the effects of Pb on cognitive function. Deficits in the Kaufman Assessment Battery for children associated with Pb in the Cincinnati cohort at 4 years of age were actually limited to those children from the poorest families (Dietrich et al. 1991). A prospective cohort study (Bellinger et al. 1989) described the particular vulnerability of children in lower SES families to the adverse effects of Pb on development, in that cognitive deficits were associated with lower BPb levels (6–7 μg/dL) in the lower SES groups than in higher SES groups. Similar deficits were reported in an Australian cohort study (Tong et al. 2000). *b*) Does environmental Pb exposure itself act on the limbic hypothalamic–pituitary–adrenal (HPA) axis, altering glucocorticoid levels and thereby enhance susceptibility to disease and dysfunction?

As these possibilities suggest, the evaluation of the effects of Pb in isolation, the focus of almost the entire experimental literature, may in actuality be far less pertinent than assessment of its interactive effects with co-occurring risk factors. Would Pb exposure have a different profile of effects when considered in the context of stress? Does Pb exposure alter responses to stress based on its associated behavioral and neurochemical effects? If stress can modulate Pb effects, then the risks associated with Pb exposure alone may be inaccurately described. The study reported here began an experimental assessment of such questions. For low SES populations, both stress and Pb exposure are lifelong events. This particular study focused on those potential contributions arising from the interaction of maternal Pb exposure and maternal stress and its long-term behavioral and neurochemical consequences for their offspring.

Materials and Methods

Animals and experimental design. Male and female Long-Evans rats were obtained from Taconic Farms (Germantown, NY, USA) for breeding. A total of four experimental groups of dams were generated: no maternal stress, no Pb exposure (NS/0, n = 14 dams), no maternal stress, Pb exposure (NS/150, n = 15 dams), maternal stress, no Pb exposure (S/0, n = 18 dams), and maternal stress plus Pb exposure (S/150, n = 23 dams).

Females began exposure to either 0 or 150 ppm Pb acetate in drinking water 2 months before breeding, and this exposure continued throughout lactation. In the absence of a prior model for this exposure protocol, this Pb exposure concentration was chosen as the best estimate of a level that would produce BPb concentrations of 20–25 μg/dL, although observed levels were in the 30–40 μg/dL range. Only a single concentration of Pb was used in these studies given the number of offspring and support time and equipment involved in examining the interactive effects of Pb with stress. Initiation of Pb exposure 2 months before breeding was used to ensure a significant body burden of Pb. After the 2 month period, males were placed with females for 5 days. Females were checked daily for the presence of a vaginal plug, and females who evidenced mating (day 1 of pregnancy) were removed and housed one per cage. Maternal stress was carried out on gestational days (GD) 16 and 17.

Litter sizes were checked at birth and culled to eight pups per litter, and pup body weights were obtained periodically thereafter. Subsets of dams and offspring were sacrificed at postnatal day (PN)1 and at day 21 for determinations of BPb levels (dams only) and for determinations of neurotransmitter levels in frontal cortex, striatum, and nucleus accumbens (day 21 only for offspring).

Pups were housed with dams and littermates until day 21, after which they were weaned and housed one per cage for behavioral experiments. Pups were given unrestricted access to food and tap water until reaching approximately 55 days of age. At this time, caloric intake was regulated until body weights reached 300 g for males or 220 g for females and thereafter stabilized at that level through restricted caloric intake.

Long-term behavioral effects were assessed using a fixed-interval (FI) schedule of food reinforcement, a behavioral baseline we have repeatedly demonstrated to be sensitive to Pb exposure (Cory-Slechta and Weiss 1985; Cory-Slechta et al. 1983, 1985). This was preceded by locomotor activity testing (not shown) in both sexes when male offspring were 60 days of age and females were 80–100 days of age. Male offspring began behavioral training at approximately 3 months of age on the FI schedule, with behavioral sessions carried out 5 days per week over a period of 20 weeks. One FI session was preceded by a 45 min restraint stress during week 9, by a challenge with the DA D_2 receptor agonist quinpirole during week 15, and by a 15 min intruder stress in the home cage during week 20. Females began FI testing later, at approximately 4 months of age, and this continued for 22 weeks. One FI session was preceded by a 45 min restraint stress before session 80, by a locomotor activity session stress before session 92, and by a 20 min cold stress (4°C) before session 105. Males and females were sacrificed 7 days and 4 days, respectively, after the final FI session.

Apparatus. FI performance was measured in operant chambers (model E10-10; Coulbourn Instruments, Inc., Allentown, PA) housed in individual sound-attenuated boxes equipped with three levers located 9 cm above the grid floor. Only the right lever was active in these experiments. Reinforcement consisted of the delivery of 45 mg food pellets (P.J. Noyes, Inc., Lancaster, NH). Behavioral responses were programmed and recorded using the SKED-11 operating system (Snapper et al. 1982) on a PD-11 Digital Equipment computer (Digital Equipment Corporation, Maynard, MA) with a resolution of 10 msec.

Behavioral procedures. Lever press responding was shaped in automated overnight sessions, after which a 1-min FI schedule of reinforcement was imposed. On this schedule, a 45-mg food pellet delivery followed the first lever press response occurring at least 1 min after the preceding food delivery, with responses occurring during the 1-min interval itself having no programmed consequences. Reinforcement delivery also initiated the next 1-min FI. Sessions ended after the completion of the 1-min interval in progress 20 min after the session began, or after a total of 22 min, whichever occurred first. Behavioral sessions were carried out 5–6 days per week between 1000 and 1700 hr.

FI performance measures. The following performance measures were computed from each session for every animal: *a*) overall response rate, or total number of responses divided by total session time; *b*) mean postreinforcement pause time, that is, the mean time to the occurrence of the first response in an interval; and *c*) mean running rate, that is, the rate of responding calculated with the postreinforcement pause time subtracted out.

Pb exposure. Pb acetate was dissolved in distilled deionized water for drinking solutions and prepared fresh on a weekly basis as in our previous studies.

Maternal stress. For maternal stress, we imposed restraint stress on GD16 and GD17. Dams assigned to stress groups were placed in restraining devices (IITC restrainer model 81 for rodents 250–400 g; 63 mm; 2.5 in.; IITC Life Sciences, Woodland Hills, MA) for 45 min three times on each of these 2 days at 900, 1200, and 1500 hr in a modification of the model of Ward and Weisz (1984). This stress paradigm was chosen because it targets development of key brain structures, including hypothalamic nuclei, hippocampus, striatum, and frontal cortex (Weinstock et al. 1998), and because of its relatively robust effects and its reported association with changes in

mesolimbic DA systems (Alonso et al. 1994; Henry et al. 1995; Takahashi et al. 1992). Control pregnant females (nonstress groups) were left undisturbed in their home cages.

Corticosterone measurement. Blood was taken by tail nicks from dams for determination of stress-induced corticosterone levels immediately after the third restraint stress on the first stress day (GD16). To avoid differences in corticosterone levels due to circadian rhythms, blood samples were collected no later than 1530 hr. We chose GD16 rather than GD17 because repeated stress can invoke adaptation that might obscure group differences (Haile et al. 2001; Orsini et al. 2002).

For offspring, blood was taken for determination of basal corticosterone levels before the imposition of a first adult stress challenge. For males, this occurred during week 9 of adult behavioral testing, and for females during week 16. The latter reflected the longer time period necessary for FI performance of females to stabilize. Corticosterone was measured by competitive enzyme immunoassay using a rabbit polyclonal corticosterone antibody (Octeia Corticosterone; Alpco Diagnostics, American Laboratory Products, Windham, NH). Sensitivity of the assay is 0.23 ng/mL.

Determinations of monoamine levels. Levels of DA, the associated metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), serotonin (5-HT), and the intracellular DA turnover (DOPAC/DA) were determined in frontal cortex, nucleus accumbens, and frontal cortex using methods established in our laboratory (Thiruchelvam et al. 2000) and measured at a sensitivity of 2 nA for striatum and frontal cortex and 1 nA for nucleus accumbens. These neurotransmitters have demonstrated sensitivity to Pb (Cory-Slechta et al. 1992, 1996; Kala and Jadhav 1995; Pokora et al. 1996) and to stress (Armando et al. 2003; Bland et al. 2003; Chou-Green et al. 2003; Finlay and Zigmond 1997; Hanley and Van de Kar 2003; Mangiavacchi et al. 2001; Scheggi et al. 2002). DA and 5-HT play key roles in the brain regions examined, which include components of both the nigrostriatal and mesolimbic DA systems, thus also allowing some assessment of specificity of effects. Specifically, after rapid decapitation, sections were dissected out and placed in 0.1 N perchloric acid. Tissues were sonicated and centrifuged for 20 min at 10,000 × *g*. The supernatants were stored at –80°C until analyzed by HPLC with electrochemical detection. The pellets were digested in 1 mL 0.5 N NaOH for measurements of protein concentration using Bio-Rad assay reagents (Bio-Rad, Hercules, CA). Concentrations of neurotransmitters are expressed as nanograms per milligram protein.

Blood lead levels. BPb levels were analyzed using anodic stripping voltammetry according to previously described methods (Cory-Slechta et al. 1987; Widzowski and Cory-Slechta 1994). The limit of sensitivity of the assay is 5 µg/dL.

Statistical analyses. No more than one male and one female from any litter were included in analyses for behavioral, neurochemical, and corticosterone effects. FI performance was analyzed using repeated-measures analyses of variance (ANOVA). When significant main effects or interactions were determined ($p \leq 0.05$), the nature of the effect was further evaluated using one-way ANOVAs, post hoc tests, or both, as appropriate. Statistical analyses of nonbehavioral end points were done using ANOVAs with consequent post hoc testing when determined by appropriate significance levels.

Principal component analyses (SPSS for Windows; SPSS, Inc., Chicago, IL) were undertaken to begin to explore relationships between basal corticosterone levels, adult neurochemical changes, and behavioral performance. These were carried out separately for each sex and for each treatment group based on eigenvalues > 1 and varimax rotation. Given that a number of different models could be tested using such approaches, for these initial analyses we examined basal corticosterone levels and mean overall response rates and postreinforcement time values from week 8 (a time point when treatment-related effects were evident in both sexes) in conjunction with those neurochemical measures that showed significant sex-related changes in response to Pb alone, stress alone, or Pb plus stress in the HPLC determinations carried out post FI testing. Basal corticosterone levels rather than stress-induced corticosterone levels were used because changes in basal FI performance and neurotransmitter levels in brain were being examined.

Results

Effects in dams. **BPb concentrations.** BPb levels of dams were determined either at PN1 or when offspring were 21 days of age (final day of Pb exposure of dams). Although Pb exposure elevated BPb levels in dams ($p < 0.0001$), there were no statistically significant differences in relation to stress or to day of sacrifice (PN1 vs. day 21). Mean ± SE BPb levels of controls dams (NS/0) and stress-only dams (S/0) were below detection limits (5 µg/dL), whereas values for the Pb groups (NS/150 and S/150) were 32.6 ± 4.4 and 40.3 ± 2.1 µg/dL at PN1 and 42.7 ± 4.0 and 38 ± 3.3 µg/dL at day 21, respectively.

Corticosterone levels. Dam corticosterone levels were elevated both in response to Pb exposure [$F(1,55) = 6.24$, $p = 0.016$] and to stress [$F(1,55) = 38.51$, $p < 0.0001$], as shown in Figure 1. Group mean values increased in the groups in the order NS/0, NS/150, S/0, and S/150, with elevations of 61%, 129%, and 161%, respectively, compared with the NS/0 group mean. Later post hoc comparisons indicated that the only two groups not exhibiting significant differences from each other were the two stress groups, S/0 and S/150 (all *p*-values < 0.05).

Neurotransmitter levels. Levels of neurotransmitters from groups of dams sacrificed at offspring PN1 and day 21 were measured in frontal cortex, nucleus accumbens, and striatum. Results from PN1 assessments, those most closely linked in time to the restraint stresses, are shown in Figure 2, where the most pronounced changes were seen in frontal cortex (Figure 2A) and nucleus accumbens (Figure 2B), with only minor effects in striatum (Figure 2C). In frontal cortex (Figure 2A), stress per se appeared to have the predominant influence at PN1, with significant decreases in DA and DOPAC in the S/0 and S/150 groups relative to the NS/0 and/or to the NS/150 groups. Concurrently, DA turnover was increased, although post hoc tests showed that this effect was restricted to the S/150 group, where levels significantly exceeded those of all other groups by values ranging from 49% to 132% [main effect of Pb: $F(1,27) = 4.27$, $p = 0.048$; main effect of stress: $F(1,27) = 8.79$, $p = 0.006$]. By day 21 (26 days after restraint stress), no residual influences of either Pb or stress on neurotransmitter levels could be detected in frontal cortex (not shown).

Changes in PN1 nucleus accumbens neurotransmitter levels were exclusively restricted to the S/150 group, with marginal increases in levels of DOPAC [64%; interaction of Pb and stress: $F(1,26) = 3.54$, $p = 0.071$] and significant increases in HVA [37%; interaction of Pb and stress: $F(1,26) =$

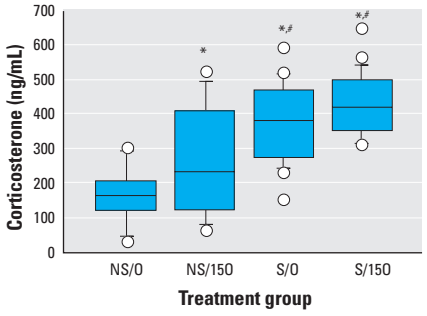

Figure 1. Group mean restraint-stress–induced corticosterone levels (ng/mL) in dams in relation to treatment conditions (NS/0, $n = 10$; NS/150, $n = 12$; S/0, $n = 18$; S/150, $n = 19$). The top and bottom of each box represent the 25th and 75th percentiles of the values within each group; the horizontal line within the box represents the group median value; the whiskers extend to the 10th and 90th percentiles of the values, and the circles outside these ranges show individual values extending beyond these parameters.
*Significantly different from the NS/0 group. #Significantly different from the NS/150 group.

5.62, $p = 0.026$]. Marginal reductions in 5-HT were also noted [–36%; main effect of stress: $F(1,25) = 4.15$, $p = 0.052$; interaction of Pb and stress: $F(1,25) = 3.74$, $p = 0.065$]. Residual changes were still evident at day 21 in nucleus accumbens.

The only change seen in striatum at PN1 was, as in nucleus accumbens, confined to the S/150 group, where increases in DOPAC levels of 57% were noted [main effect of Pb: $F(1,30) = 5.91$, $p = 0.021$]. An increase in DA turnover in response to Pb per se was detected in statistical analyses [$F(1,30) = 4.73$, $p = 0.038$], although neither Pb group alone (NS/150, S/150) was found to differ from other groups in later post hoc tests. By day 21, there were no residual changes in DA or its metabolites, turnover, or 5-HT in striatum.

Effects in offspring. **Body and tissue weight and litter parameters.** Tables 1–3 report wet weights of various brain regions, total numbers of pups per litter, and associated body weights for offspring at PN1 and day 21, and body weights at 2 months of age (before FI performance testing). For pups sacrificed at PN1 (Table 1), there were no detectable changes in response to Pb or stress or any interactions with respect to pup whole-brain weight. Only one effect related to litter size was found in which total numbers of pups per litter was slightly increased in the S/150 group [interaction of Pb × stress: $F(1,32) = 5.47$, $p = 0.026$].

At 21 days of age (Table 2), hippocampal weights of male offspring were increased about 12–13% in response to stress [$F(1,26) = 6.74$, $p = 0.015$] relative to nonstressed controls (NS/0). In female offspring, differential effects of Pb and stress on cerebellar weights were found [Pb × stress interaction: $F(1,28) = 5.76$, $p = 0.023$], with weights reduced by stress alone (S/0 vs. NS/0) and Pb alone (NS/150 vs. NS/0) but not by the combination. Female offspring also showed increases of approximately 7–10% in kidney weights in response to Pb exposure [$F(1,28) = 4.4$, $p = 0.045$] relative

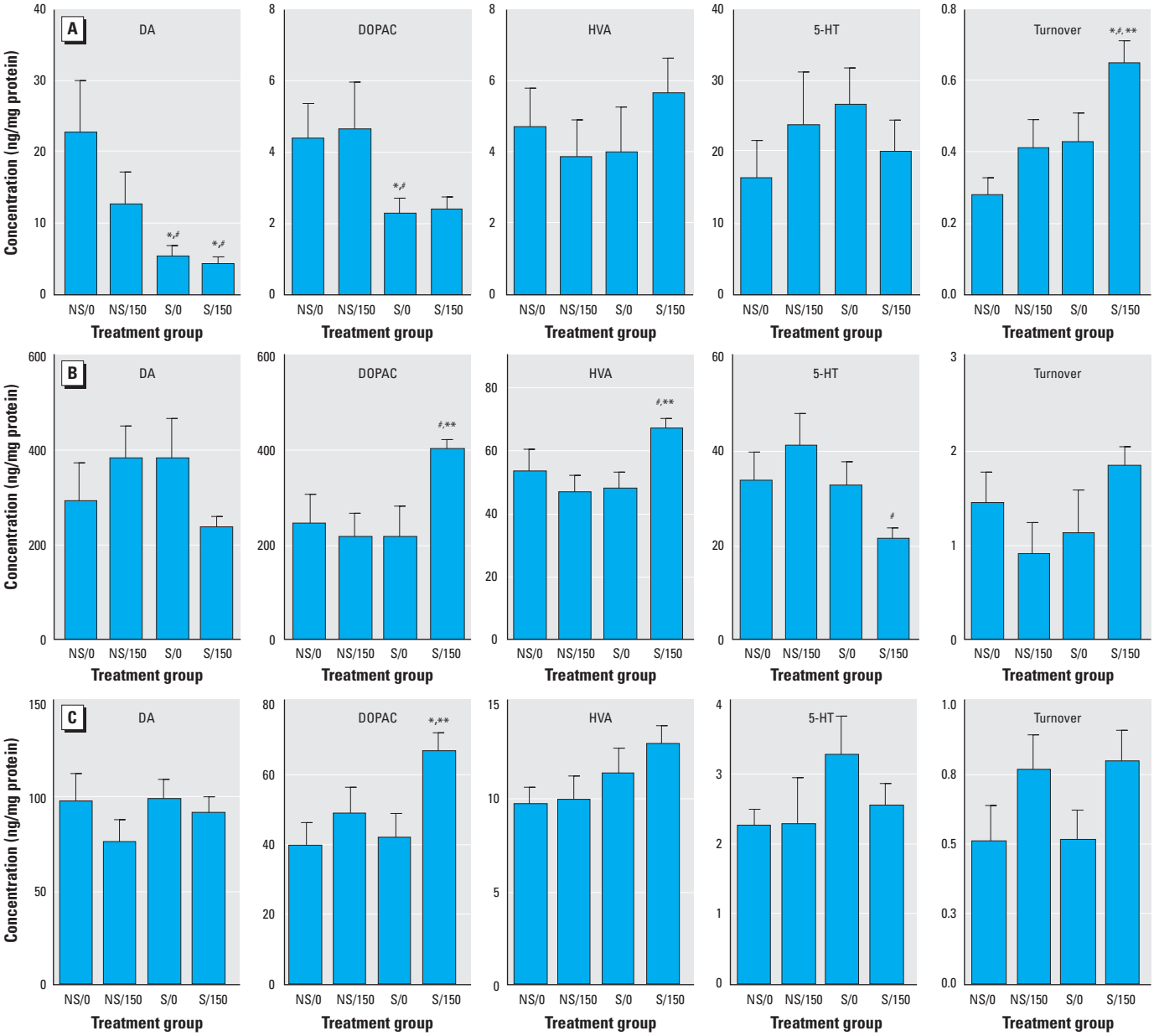

Figure 2. Group mean ± SE concentrations of DA, DOPAC, HVA, and 5-HT (ng/mg protein) and DA turnover in dams in frontal cortex (*A*; $n = 5$–11/group), nucleus accumbens (*B*; $n = 7$–10/group), and dorsal striatum (*C*; $n = 6$–12/group) taken at PN1.
*Significantly different from the NS/0 group. #Significantly different from the NS/150 group. **Significantly different from the S/0 group.

to the NS/0 group. There were no group differences in average litter weights at 21 days of age. Although there were significant sex differences in body weights at 2 months of age (Table 3), there were no differences related to either Pb exposure or stress.

Basal corticosterone levels. Effects on basal corticosterone levels, determined before the first adult stress in offspring, differed by both sex and treatment (Figure 3). Pb exposure per se markedly increased corticosterone levels in males [Figure 3A; $F(1,28) = 7.36$, $p = 0.011$] with an increase of 97% relative to the NS/0 group. Maternal stress alone tended to decrease basal corticosterone levels in male offspring, but these effects were not statistically significant. Pb exposure still increased basal corticosterone levels in S/150 males because there was no significant interaction of Pb × stress, and basal corticosterone levels of the S/150 group were 110% greater than those of the S/0 group and 48% higher than the NS/0 group.

Pb exposure likewise increased basal corticosterone levels in female offspring [Figure 3B; main effect of Pb: $F(1,34) = 5.03$, $p = 0.0032$], but only in combination with stress, as indicated in later post hoc tests. Values of the NS/150 group exceeded, although not significantly, those of the NS/0 group by 27%. Corticosterone levels of the S/150 group, however, were significantly greater than those of both the NS/0 group (58%; $p = 0.05$) and the S/0 group (61%; $p = 0.03$).

Day 21 neurotransmitter changes. Determinations of levels of DA and its metabolites, DA turnover, and 5-HT in frontal cortex and striatum (combined dorsal and ventral striatum at this age) are shown in Figures 4 and 5, respectively, and summarized in Table 4. In frontal cortex (Figure 4), changes were found primarily in female offspring, where Pb exposure per se reduced levels of DA [main effect of Pb: $F(1,25) = 4.77$, $p = 0.039$] with general decreases in the NS/150 and S/150 groups relative to the NS/0 and S/0 groups, because no specific post hoc comparisons were statistically significant. These reductions ranged from 40% to 62%. HVA was altered in response to stress [main effect of stress: $F(1,13) = 10.19$, $p = 0.007$], again with nonspecific increases, ranging from 25% to 175%, in the S/0 and S/150 groups relative to the NS/0 and NS/150 groups. For male offspring, the only changes seen were in levels of DOPAC, and these were attributable to stress [$F(1,25) = 5.8$, $p = 0.024$], deriving from marginally significant increases, ranging from 77% to 134%, in the S/150 group relative to all other groups ($p = 0.074$, 0.077, and 0.088 for the NS/0, S/0, and NS/150 comparisons, respectively).

In combined dorsal striatum and nucleus accumbens (Figure 5), changes in neurotransmitter levels were seen exclusively in female offspring, in levels of DA and its metabolites and DA turnover, but not in levels of 5-HT. DA levels were reduced by Pb exposure [$F(1,28) = 13.15$, $p = 0.001$], and DA turnover correspondingly increased [$F(1,27) = 7.12$, $p = 0.013$]. Relative to control (NS/0), DA levels were reduced in the NS/150 and S/150 groups by 33% and 44%, respectively ($p = 0.023$ and 0.01). Levels of the S/150 group were also reduced by 37% relative to the S/0 group. Increases in DA turnover were seen in both Pb groups, as well, but were statistically significant only in the S/150 group, where values exceeded those of the NS/0 and S/0 groups by approximately 84% in both cases ($p = 0.007$ and 0.004, respectively). The similar changes observed in DOPAC and HVA were jointly influenced by Pb exposure and stress [Pb × stress interaction: DOPAC: $F(1,27) = 5.96$, $p = 0.022$; HVA: $F(1,27) = 13.12$, $p = 0.001$]. In both cases, the greatest reductions relative to NS/0 were seen in response to Pb exposure alone (NS/150; $p = 0.008$ and 0.0003, respectively). For HVA, the two stress groups also exhibited significantly lower levels than did controls (S/0 and S/150; $p = 0.012$ and 0.0049, respectively). Stress appeared to modulate these effects of Pb, however, particularly for HVA, where levels of the NS/150 group were still significantly lower than those of the S/150 group ($p = 0.02$).

FI performance. Performance of male offspring on the FI schedule of reward across 20 weeks of testing is shown in Figure 6. Response rates of the NS/150 group were considerably lower than those of all other groups, particularly across the first half of the testing protocol [main effect of Pb: $F(1,28) = 5.48$, $p = 0.027$]. By week 8, this amounted to a 47% decrease relative to the NS/0 group. The decrease in overall response rate appeared to be largely due to corresponding changes in postreinforcement pause time across groups [interaction of Pb × week: $F(18,414) = 3.51$, $p < 0.001$; interaction of Pb × stress by week: $F(18,414) = 5.00$, $p < 0.001$], with the NS/150 group showing longer postreinforcement pause time values, particularly across the first 8 week period (before the testing of pre-FI session stressors), with values ranging from 125% to 224% of control. Similar effects were seen in the S/150 group at weeks 5, 6, and 7, contributing to the interaction effects in the statistical analyses. Maternal stress alone (S/0) did not affect rates or postreinforcement pause time values. By week 12, and after the introduction of adult stressors, rates and postreinforcement pause times of the groups had largely converged.

A very different pattern of outcomes was observed in female offspring (Figure 7), where the major impact on FI performance resulted from stress alone, resulting in a clear Pb × stress interaction in the statistical analyses [$F(1,32) = 5.8$, $p = 0.022$]. Overall response rates of the S/0 group exceeded those of controls by values ranging from 133% to 227% and remained elevated across all weeks of testing [Pb × week: $F(15,480) = 1.72$, $p = 0.044$]. In contrast, overall response rates of the NS/150 and the S/150 groups did not differ

Table 1. Litter sizes and offspring body and brain weights on PN1 (n = 7–13).

Treatment group	Mean body weight (g)		No. of pups			Pup brain weight (mg)
	Male	Female	Male	Female	Total	
NS/0	6.2 ± 0.15	5.9 ± 0.18	5.7 ± 1.0	3.6 ± 0.6	10.1 ± 0.6	226.8 ± 10.7
NS/150	6.0 ± 0.29	5.6 ± 0.27	4.1 ± 0.48	4.4 ± 0.9	8.0 ± 2.1	228.6 ± 9.2
S/0	6.3 ± 0.23	5.5 ± 0.22	4.7 ± 0.44	5.9 ± 0.9	8.4 ± 1.5	235.5 ± 6.2
S/150	6.0 ± 0.25	5.5 ± 0.17	4.5 ± 0.6	4.1 ± 0.7	12.1 ± 0.6	221.3 ± 5.7

Table 3. Offspring body weight at 2 months of age (n = 7–11).

Treatment group	Mean body weight (g)	
	Male	Female
NS/0	268.8 ± 11.4	174.6 ± 6
NS/150	258.2 ± 18	174.6 ± 9
S/0	271.9 ± 10.5	178.8 ± 3.8
S/150	263.8 ± 9.9	172.8 ± 5.3

Table 2. Offspring body, regional brain, and kidney weights at 21 days of age (n = 6–10).

Treatment group	Males					Females				
	Mean body weight (g)	Striatum (mg)	Hippocampus (mg)[a]	Cerebellum (mg)	Kidney (mg)	Mean body weight (g)	Striatum (mg)	Hippocampus (mg)	Cerebellum (mg)[b]	Kidney (mg)[b]
NS/0	41.9 ± 1.2	12.1 ± 0.9	94.0 ± 8.8	168.2 ± 8.7	247.2 ± 19.9	41.8 ± 1.2	12.8 ± 1.5	98.5 ± 9.9	171.3 ± 6.0	241.9 ± 10.7
NS/150	41.6 ± 1.9	12.5 ± 1.1	84.9 ± 6.6	167.8 ± 7.0	256.2 ± 16.5	41.5 ± 1.8	11.3 ± 1.3	98.7 ± 8.4	156.4 ± 5.1	258.1 ± 12.5
S/0	41.0 ± 1.0	12.2 ± 1.1	105.2 ± 5.2	172.4 ± 3.6	244.0 ± 13.2	38.5 ± 1.4	13.6 ± 1.3	95.3 ± 4.8	153.8 ± 6.8	224.5 ± 12.3
S/150	41.1 ± 1.5	13.2 ± 1.5	106.2 ± 4.9	163.9 ± 6.1	257.4 ± 12.6	40.5 ± 1.1	11.5 ± 0.9	95.7 ± 3.0	169.6 ± 6.0	266.0 ± 16.4

[a]Main effect of stress. [b]Main effect of Pb.

from control. As with males, the changes in overall response rates were a function of alterations in postreinforcement pause time values, with run rates (not shown) not altered by treatments. Postreinforcement pause times of the S/0 group were significantly shorter than those of all other groups, and values changed differentially across the course of testing in relation to both Pb exposure and stress [Pb × stress × weeks: $F(15,480) = 2.06$, $p = 0.011$]. For the S/0 group, postreinforcement pause time values were generally reduced by approximately 25%. The statistical interaction most likely reflects the gross differences in postreinforcement pause times occurring during the first week of testing that differentiate all four treatment groups.

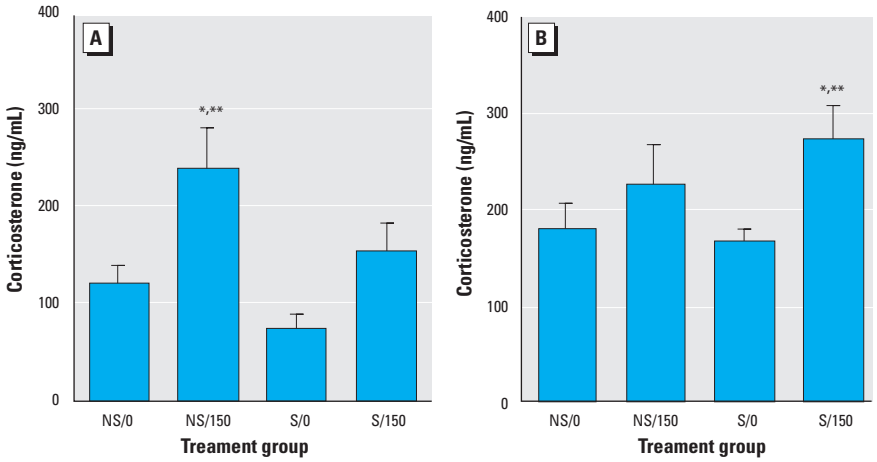

Figure 3. Group mean ± SE basal corticosterone concentrations (ng/mL) of male (*A*; $n = 7$–9/group) and female (*B*; $n = 8$–11/group) offspring.
*Significantly different from the NS/0 group. **Significantly different from the S/0 group.

Adult neurotransmitter changes. Despite trends in the data, there were no residual changes in catecholamines in frontal cortex of male offspring (Figure 8A; summarized in Table 4). However, interactions of Pb and stress were evident in nucleus accumbens (Figure 8B), in levels of DA [$F(1,28) = 4.51$, $p = 0.043$], DOPAC [$F(1,28) = 6.84$, $p = 0.014$], and 5-HT [$F(1,28) = 4.29$, $p = 0.048$]. For DA and DOPAC, Pb exposure alone was generally without effect relative to control (NS/0), stress alone tended to reduce levels, and combined Pb plus stress increased levels relative to control and to stress alone. These effects were statistically significant in post hoc tests for DOPAC but not DA. Pb exposure increased both HVA concentrations and DA turnover [$F(1,28) = 25.87$, $p < 0.001$ and $F(1,28)$

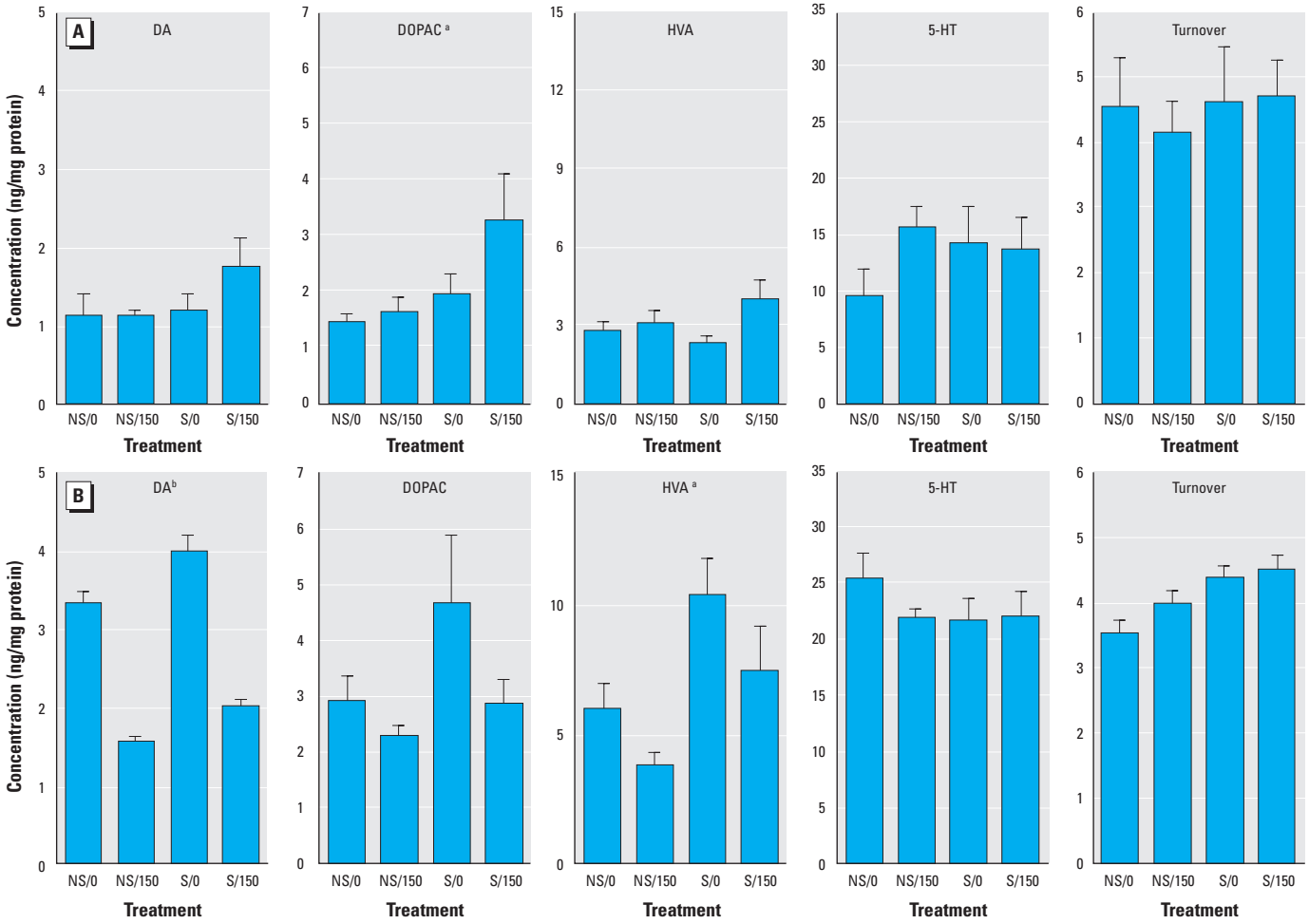

Figure 4. Group mean ± SE concentrations of DA, DOPAC, HVA, and 5-HT (ng/mg protein) and DA turnover in frontal cortex of male (*A*; $n = 5$–8/group) and female (*B*; $n = 3$–9/group) offspring determined at 21 days of age.
[a]Main effect of stress. [b]Main effect of Pb.

= 6.46, $p = 0.017$, respectively], and stress alone decreased HVA concentrations [$F(1,28) = 10.13$, $p = 0.004$] relative to groups receiving Pb (Figure 8B). In the case of 5-HT, Pb exposure alone increased levels relative to controls, whereas no changes were found in either the S/0 or S/150 groups. In contradistinction to the nucleus accumbens, residual changes in striatal levels of catecholamines were generally related exclusively to stress. This included marked increases (56%) in DA [$F(1,45) = 58.4$, $p < 0.0001$], and reductions (65%) in DOPAC [$F(1,45) = 186.0$, $p < 0.0001$] and DA turnover [73%; $F(1,45) = 162.4$, $p < 0.0001$] (Figure 8C). Increases in 5-HT (44%), however, were seen only under condition of Pb plus stress [$F(1,45) = 9.6$, $p = 0.003$].

In female offspring as adults (Figure 9, Table 4), the pattern of neurotransmitter system changes differed notably from that of males. First, interactions of Pb plus stress

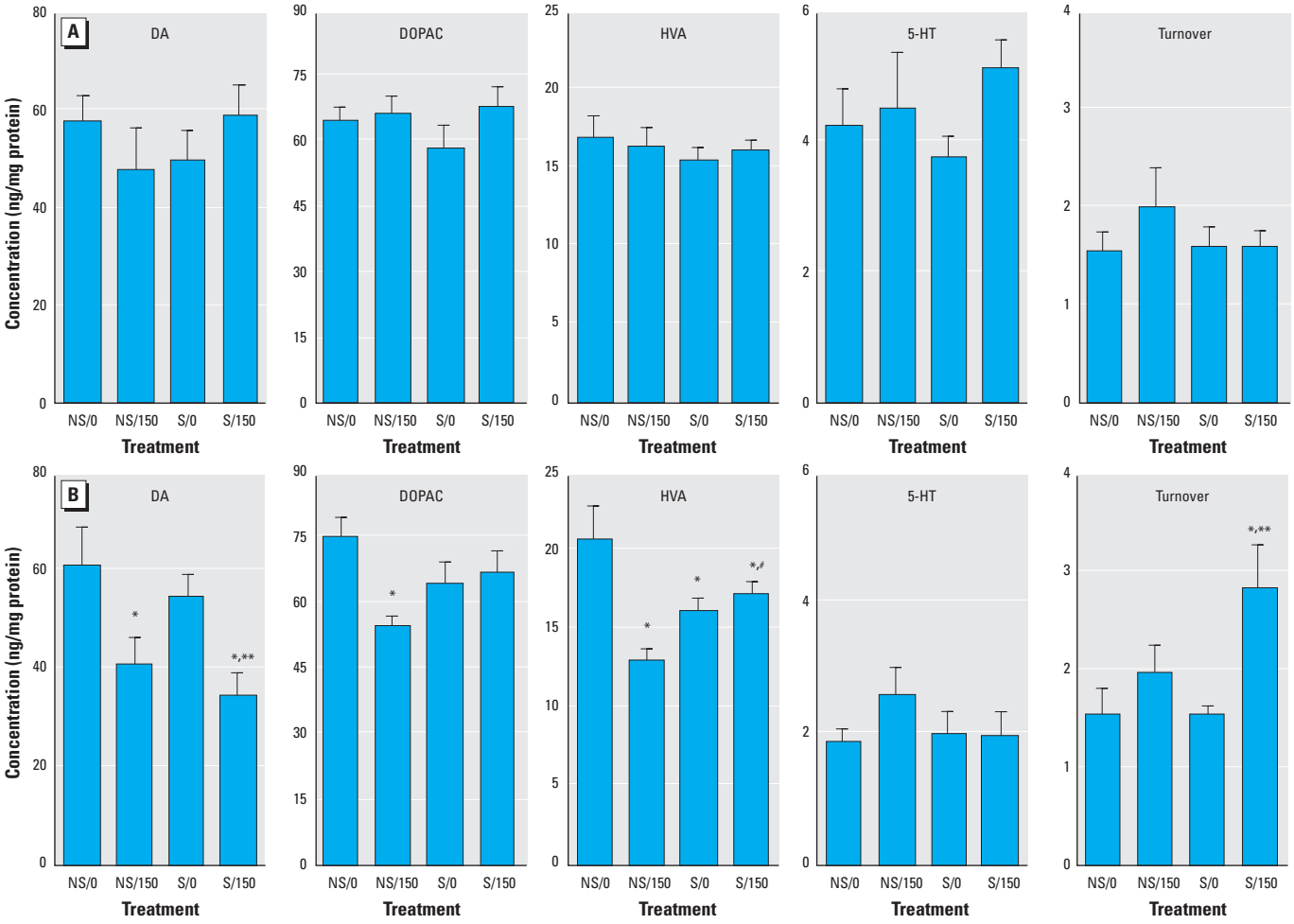

Figure 5. Group mean ± SE levels of DA, DOPAC, HVA, and 5-HT (ng/mg protein) and DA turnover in striatum (combined dorsal striatum and nucleus accumbens) of male (*A*; $n = 4$–9/group) and female (*B*; $n = 6$–9/group) offspring determined at 21 days of age.
*Significantly different from the NS/0 group. #Significantly different from the NS/150 group. **Significantly different from the S/0 group.

Table 4. Summary of neurochemical changes in offspring.

Sex, tissue	Age	DA	DOPAC	HVA	5-HT	Turnover
Males						
Frontal cortex	21 days	NC	NC	NC	NC	NC
	Adult	NC	NC	NC	NC	NC
Nucleus accumbens	21 days	ND	ND	ND	ND	ND
	Adult	NC	↓S; ↑Pb + S[a]	↑Pb; not Pb + S[a]	↑Pb; not Pb + S[a]	~↑Pb; ~↑Pb + S
Striatum	21 days	NC	NC	NC	NC	NC
	Adult	↑Pb; ↑Pb + S	↓S; ↓Pb + S	NC	↑Pb + S[b]	↓S; ↓Pb + S
Females						
Frontal cortex	21 days	↓Pb; ↓Pb + S	NC	↑S; ↑Pb + S	NC	NC
	Adult	↑Pb + S[b]	↑S; ↑Pb + S	ND	NC	↓Pb; ↓Pb + S
Nucleus accumbens	21 Days	ND	ND	ND	ND	ND
	Adult	↓Pb; ↓Pb + S	NC	↑Pb; not Pb + S[a]	↓Pb; ↓Pb + S	NC
Striatum	21 days	↓Pb; ↓Pb + S	↓Pb; not Pb + S[a]	↓Pb; ↓S; ↓Pb + S	NC	↑Pb + S[b]
	Adult	↑Pb; not Pb + S[a]	↑Pb; ↑S; not Pb + S[a]	↑S; not Pb + S[a]	~↓Pb + S[b]	↑Pb; ↑S; not Pb + S[a]

Abbreviations: ~, marginal; NC, no change; ND, not determined.
[a]Modulation of Pb and/or stress-alone effect by Pb plus stress. [b]Pb plus stress effects with no effect of Pb alone or stress alone.

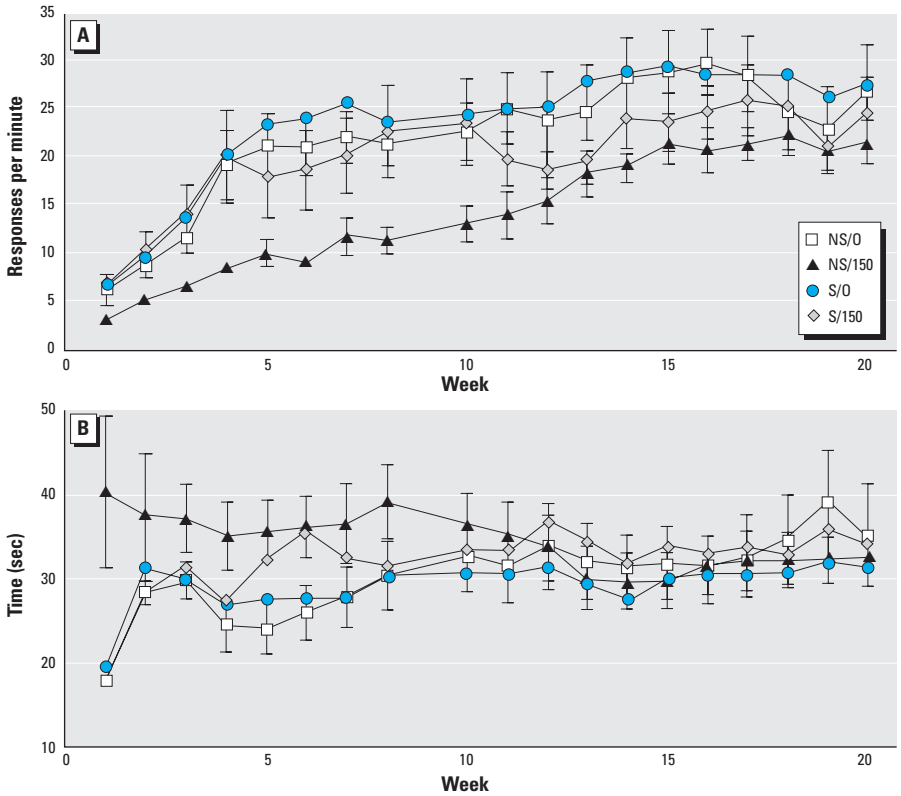

Figure 6. Group mean ± SE overall response rates (*A*) and postreinforcement pause times (*B*) on the FI schedule of reinforcement for male offspring across the 20 weeks of behavioral testing. Sample sizes: NS/0 group, 7; S/0 group, 10; NS/150 group, 8; S/150 group, 8.

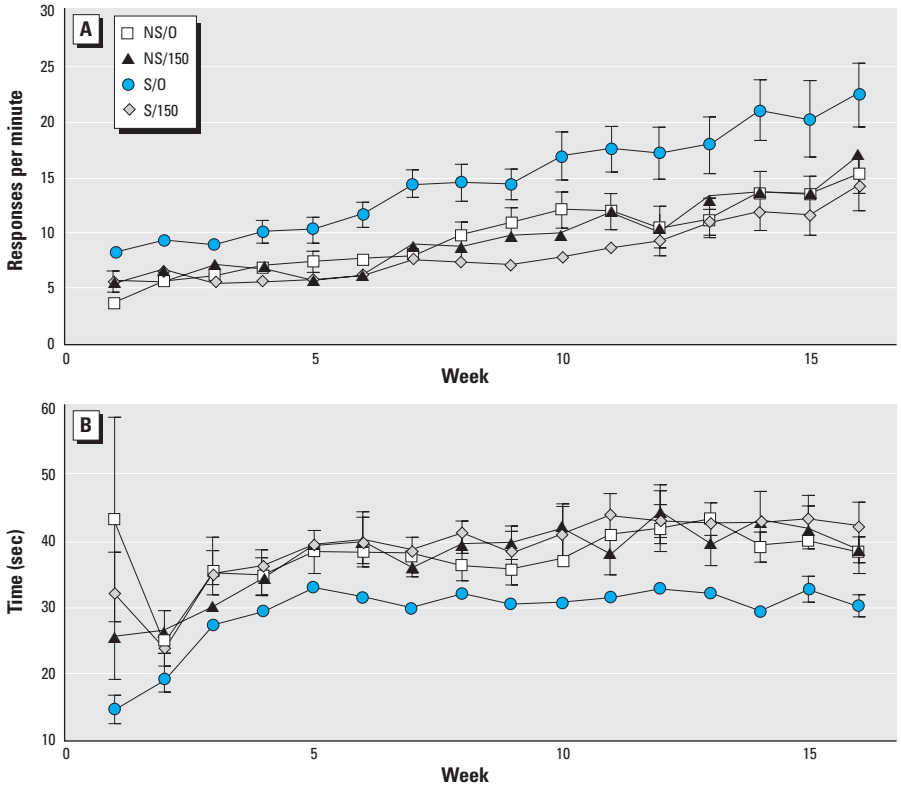

Figure 7. Group mean ± SE overall response rates (*A*) and postreinforcement pause times (*B*) on the FI schedule of reinforcement for female offspring across the 22 weeks of behavioral testing. Sample sizes: NS/0 group, 8; S/0 group, 11; NS/150 group, 7; S/150 group,10.

were found in frontal cortex (Figure 9A), as selective increases in DA [30%; Pb × stress: $F(1,32) = 6.85$, $p = 0.013$]. Other changes in frontal cortex included increases in DOPAC related to stress (48%) and Pb plus stress (67%), relative to Pb alone [Pb × stress interaction: $F(1,32) = 4.24$, $p = 0.048$], as well as Pb-related increases in DA turnover [$F(1,32) = 9.85$, $p = 0.004$]. Interactions observed in striatum were similar across all measures, namely, increases in response to Pb and stress alone, and no change in the S/150 group. For DA, Pb exposure significantly increased (33%) and stress marginally increased (25%) levels, whereas no such changes were found with Pb plus stress [Pb × stress interaction: $F(1,32) = 7.8$, $p = 0.009$]. For DOPAC, levels were increased by both Pb alone (39%) and stress alone (53%), whereas no changes were found in the S/150 group [Pb × stress interaction: $F(1,32) = 11.12$, $p = 0.002$]. For HVA, stress alone increased levels (37%), whereas no changes were detected with either Pb alone or Pb plus stress [Pb × stress interaction: $F(1,32) = 8.09$, $p = 0.008$]. Similarly, DA turnover was significantly increased by Pb alone (39%) and by stress alone (54%), but no changes were seen in the S/150 group [interaction of Pb × stress: $F(1,32) = 11.14$, $p = 0.002$]. 5-HT levels in the S/150 group were reduced relative to both the NS/150 and S/0 groups. In contrast to frontal cortex and striatum, changes observed in nucleus accumbens were generally exclusively related to Pb exposure. DA levels were decreased by Pb (63%) and by Pb plus stress (54%) [main effect of Pb: $F(1,32) = 24.5$, $p < 0.0001$]. Concurrently, increases in the levels of the metabolites DOPAC [22 and 20%, respectively; main effect of Pb: $F(1,32) = 4.89$, $p = 0.034$] and HVA [36% and 15%, respectively; main effect of Pb: $F(1,32) = 7.06$, $p = 0.012$] and in DA turnover [22% and 20%, respectively; main effect of Pb: $F(1,32) = 5.16$, $p = 0.03$] were observed. Levels of 5-HT were significantly reduced in both the NS/150 (39%) and S/150 groups [38%; main effect of Pb: $F(1,32) = 6.22$, $p = 0.018$].

Relationships among basal corticosterone levels, neurotransmitter levels, and behavioral performance. Factor loading plots showing the first three components of principal component analyses, based on mean overall response rates and postreinforcement pause time values (week 8), basal corticosterone levels, and significant neurochemical outcomes (summarized in Table 4) of male offspring, are shown separately for each of the four treatment groups in Figure 10. Relationships between these variables differed substantially across the four groups. In the NS/0 group (Figure 10A), both FI overall response rates and postreinforcement pause times clustered most closely with nucleus accumbens DOPAC and nucleus accumbens DA turnover (oval; top

left), where basal corticosterone levels clustered with striatal HVA levels and nucleus accumbens 5-HT levels (oval; bottom right). In response to maternal stress per se (S/0; Figure 10B), postreinforcement pause time clustered with basal corticosterone as well as with nucleus accumbens and striatal 5-HT levels and with striatal HVA levels; FI response rates were similarly although less closely clustered with these neurochemical measures. For the NS/150 group (Figure 10C), both FI overall response rates and postreinforcement pause time values, which differed significantly from corresponding control values (Figure 8), clustered with basal corticosterone levels, which also were increased by Pb (Figure 3). In the S/150 group (Figure 10D), FI overall response rates correlated with measures of striatal DA function, specifically striatal DOPAC, DA turnover, and 5-HT levels, whereas postreinforcement pause time values clustered most closely with basal corticosterone.

Corresponding plots for female offspring are shown in Figure 11. In addition to changes in both striatum and nucleus accumbens catecholamines in response to treatments, females also showed changes in frontal cortical neurotransmitter levels (Table 4), which were therefore included in the principal components analyses. In control females (Figure 11A), FI response rates clustered with measures of frontal cortical DA function, including DOPAC and DA turnover (oval; top right), whereas postreinforcement pause time and basal corticosterone levels were tightly clustered with striatal 5-HT levels (oval; left). As with males, Pb exposure, stress, and their combination changed these relationships. With stress alone (Figure 11B), FI response rates, which were significantly elevated relative to controls (Figure 9), were now closely associated with basal corticosterone levels (oval; right); postreinforcement pause time values tended to cluster with nucleus accumbens HVA levels (oval; center). With Pb exposure alone (Figure 11C), FI rates were associated with multiple measures of nucleus accumbens and striatal DA function (oval; top right), whereas postreinforcement pause time clustered with nucleus accumbens 5-HT (oval; top left), and basal corticosterone showed no obvious associations (oval; bottom left). Under conditions of combined Pb plus stress (Figure 11D), both rate and postreinforcement pause time clustered with nucleus accumbens DA and striatal DOPAC and DA turnover, whereas basal corticosterone associated with striatal DA, HVA, nucleus accumbens HVA, and frontal cortical HVA.

Discussion

In this study we sought to examine the hypothesis that stress and Pb exposure, which co-occur as environmental risk factors and share common central nervous system targets, would interact such that effects of either risk factor alone could differ in the presence of the other. Both Pb exposure and environmental stress are lifelong events. This experiment, however, focused specifically on contributions arising maternally for both stress and Pb exposure and the resulting consequences for offspring. Maternal restraint stress was imposed on GD16 and GD17 of gestation, a period of key brain structure development, including hypothalamic nuclei, hippocampus, striatum, and frontal cortex (Weinstock et al. 1998), and occurred in conjunction with Pb exposure that began 2 months before

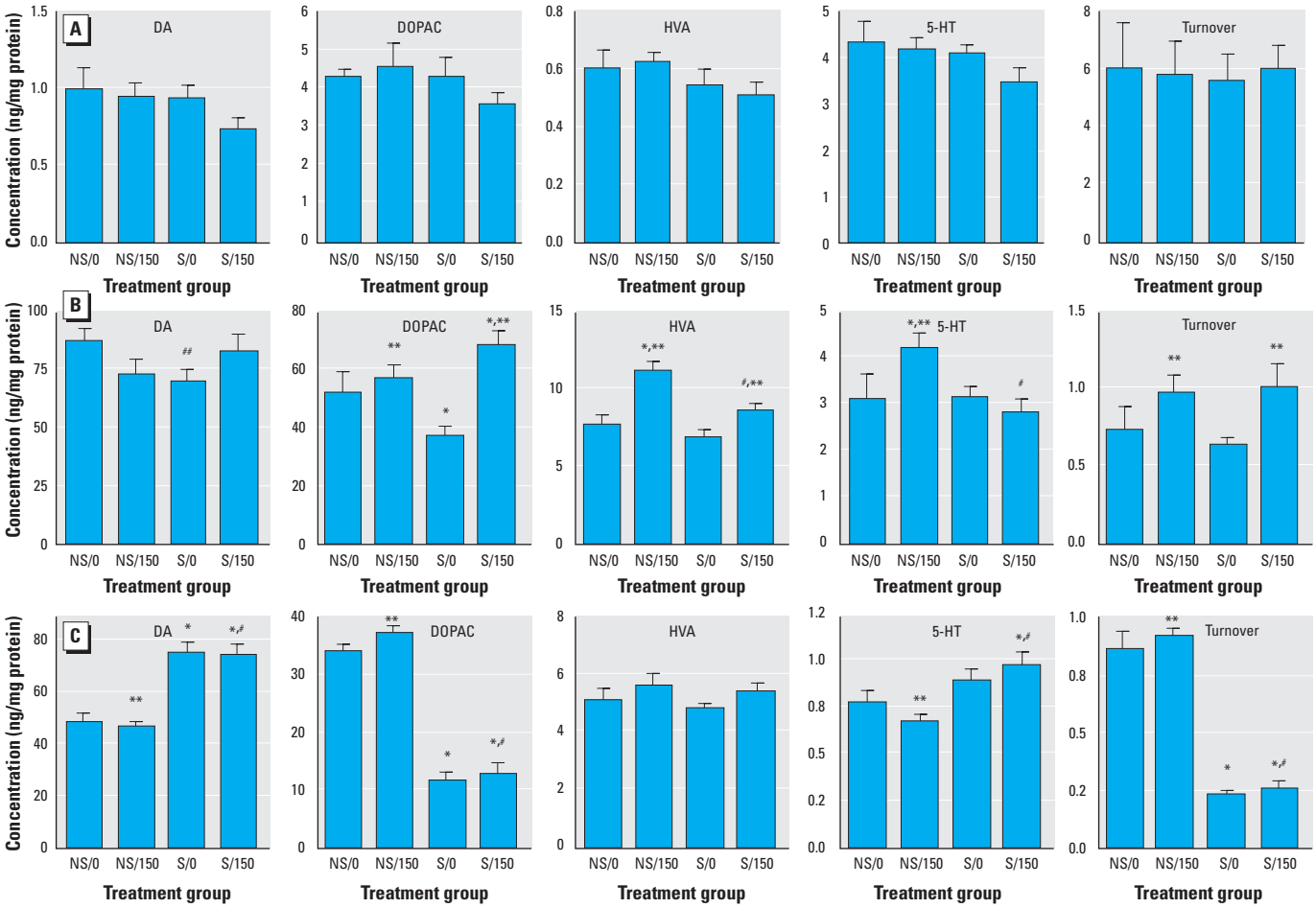

Figure 8. Group mean ± SE concentrations of DA, DOPAC, HVA, and 5-HT (ng/mg protein) and DA turnover in frontal cortex (*A*), nucleus accumbens (*B*), and striatum (*C*) of male offspring measured after the termination of behavioral testing. Sample sizes: NS/0 group, 7; S/0 group, 10; NS/150 group, 8; S/150 group, 8.
*Significantly different from the NS/0 group. #Significantly different from the NS/150 group. **Significantly different from the S/0 group. ##Marginally different from the NS/0 and S/150 groups.

breeding and continued throughout lactation, with offspring followed into adulthood. The validity of the stress paradigm employed in this study was confirmed by the increases in corticosterone levels of stressed dams (Miller and Chernoff 1995; Monteiro et al. 1989) and corresponding changes in mesocorticolimbic DA function, particularly in the nucleus accumbens and prefrontal cortex.

Several notable findings emerged from these experiments. These include confirmation of the hypothesis that Pb and stress interact. Moreover, for some measures, effects were observed only when Pb and stress were combined. Furthermore, not only did the consequences of preweaning Pb alone, maternal stress alone, and their combination differ markedly by sex, but also each had permanent consequences for offspring, which manifest variously as changes in neurochemical measures, corticosteroid systems, and/or behavior. Collectively, these findings also indicate that the interactions of Pb and stress are not easily predictable, which has significant implications for human health risk assessment, and are not surprising given the wide variety of effects each variable has alone when considered just in relation to brain function.

One particularly noteworthy finding was that developmental Pb exposure per se increased levels of corticosterone, the main glucocorticoid of the rat (Vazquez 1998). Specifically, both male and female offspring (Figure 3) showed elevated basal corticosterone levels related to Pb when measured as adults. For males, the highest levels were produced by Pb exposure per se, with stress actually slightly decreasing corticosterone levels. Importantly, for females, only offspring from dams that had been subjected to both maternal stress and Pb exposure showed elevated corticosterone levels, indicative of potentiated effects. These increases in corticosterone presumably reflect permanent changes in basal levels for both sexes because stress was strictly maternal and Pb exposure ended at 21 days of age, whereas basal corticosterone of offspring was not measured until well into adulthood. In this regard, our findings concur with those from other studies that have likewise reported permanent changes in offspring corticosterone concentrations resulting from prenatal stress (Maccari et al. 2003). Moreover, it appears that even short-term exposure can increase corticosterone, as evidenced by the elevations observed in dams measured after < 3 months of Pb exposure (Figure 1).

These findings have particular significance when one considers that the increased disease and dysfunction associated with low SES has been ascribed to chronic elevation of glucocorticoid levels. Chronically elevated glucocorticoid levels have been associated with such adverse effects as increased resistance to insulin, hypertension and hypercholesterolemia, and arteriosclerosis (Munck et al. 1984). Low SES populations are also those with the greatest levels of Pb exposure. Thus, the current findings suggest that Pb exposure, by increasing corticosterone levels, could actually act as a causative factor in this increased incidence of disease/dysfunction that has been noted in association with low SES. In this context, it is interesting that a recent study has reported increased all-cause,

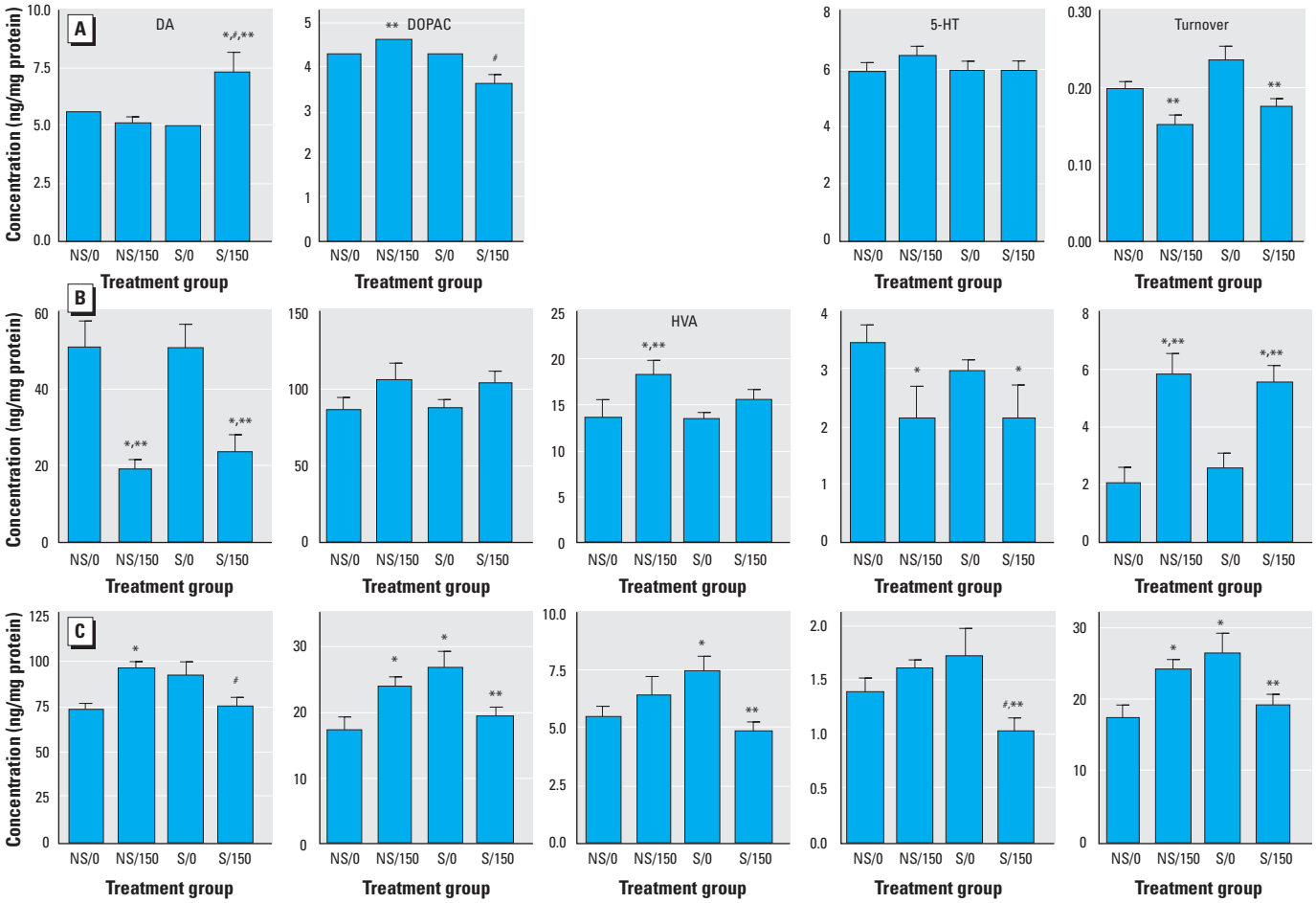

Figure 9. Group mean ± SE concentrations of DA, DOPAC, HVA, and 5-HT (ng/mg protein) and DA turnover in frontal cortex (*A*), nucleus accumbens (*B*), and striatum (*C*) of female offspring measured after the termination of behavioral testing. Sample sizes: NS/0 group, 8; S/0 group, 11; NS/150 group, 7; S/150 group, 10. HVA levels in frontal cortex were largely undetectable and therefore are not shown.
*Significantly different from the NS/0 group. #Significantly different from the NS/150 group. **Significantly different from the S/0 group.

circulatory, and cancer mortality associated with BPb levels of 20–29 µg/dL relative to populations with values < 10 µg/dL (Lustberg and Silbergeld 2002).

To date, only two other studies in rats appear to have examined the potential relationship of Pb and corticosterone. Virgolini et al. (1999) suggested that increased stress was the basis of enhanced voluntary ethanol consumption and elevated corticosterone levels in male offspring exposed gestationally and lactationally to Pb via dams with BPb concentrations of about 25 µg/dL. In another study, postnatal Pb exposure associated with BPb concentrations of approximately 70 µg/dL reduced cold swim endurance in female rats at 21 and 30 days of age and in male rats at 21 days of age. Corticosterone levels were elevated in both sexes, although only at 30 days of age (Yu et al. 1996). The present study indicates that even brief developmental exposures to Pb may produce an irreversible impact on the limbic HPA axis and associated corticosterone function. Whether this occurs directly or through Pb-associated changes in DA or glutamate in mesocorticolimbic systems is unclear (Barrot et al. 2000; McEwen 2001; Moghaddam 2002).

Protracted elevation of glucocorticoids has also been demonstrated to produce hippocampal neuronal dysfunction and even neuron loss and increased density of microglia, suggesting the ability to accelerate neuropathology associated with brain aging (Kerr et al. 1991; McEwen et al. 1968; Sapolsky et al. 1986). Also well documented is the adverse impact of elevated corticosterone levels, both acutely and chronically, on learning and memory processes (Lupien et al. 2001). Pb exposure likewise has been shown to be associated with decreases in IQ scores of children in prospective epidemiologic studies even at very low blood Pb concentrations (Canfield et al. 2003), and to selectively impair learning in experimental models (Cohn et al. 1993). Interestingly, U-shaped and inverse-U–shaped dose–effect curves have been reported for modulation of cognitive function both by corticosteroids and by Pb exposure (Davis and Svendsgaard 1990; Lupien and McEwen 1997). Thus, one new mechanistic consideration arising from the data reported here is that Pb-induced learning impairments and/or its other central nervous system effects could arise through increased corticosterone and its influence on mineralocorticoid receptors, located primarily in the septohippocampus, and/or on glucocorticoid receptors, more widely distributed throughout the brain (Joels and de Kloet 1994). Antagonists of each receptor type will be particularly useful in addressing such questions.

Three types of interactions were possible in this study: effects of Pb alone not seen in the Pb plus stress (S/150) group, effects of stress alone not seen in the Pb plus stress (S/150) group, and effects of Pb plus stress that did not occur in the Pb-alone (NS/150) or stress-alone (S/0) groups. Interactions of Pb and stress were observed in both male and female offspring. For example, in males, basal corticosterone levels are increased after exposure to Pb alone but decreased after exposure to stress alone; combined Pb plus stress resulted in effects intermediate between the two. For FI performance, we did not observe the rate-altering effects of Pb in males in the Pb plus stress (S/150) group, and similarly, we did not observe the FI-rate–increasing effects of stress alone in females in the S/150 group. Various neurochemical changes observed in both males and females in response to Pb and/or stress were not necessarily matched by corresponding changes in the S/150 group. In addition, some alterations occurred only in the presence of both risk factors, in some cases suggestive of potentiated effects. For female offspring, basal corticosterone levels did not change significantly in response to either Pb or stress alone but were notably increased (52%) by combined exposure (Figure 3). In relation to long-term neurochemical changes (Table 4), other examples include changes in striatal 5-HT levels in adult males and frontal cortical DA levels and striatal 5-HT levels in females.

The effects of Pb, stress, and Pb plus stress differed substantially in males and females, which cannot be attributed to differences in exposure, at least as indicated by BPb values. The effects of Pb exposure were more prominent than those of stress in male offspring even though important interactions were noted. For example, only Pb exposure affected behavioral performance, as shown by substantial reductions in FI rates and increases in postreinforcement pause times (Figure 8). In addition, only Pb exposure significantly elevated basal corticosterone levels in males (Figure 3), and interestingly, both rates and postreinforcement pause times were found to cluster with basal corticosterone in the principal component analysis for this group and not for other treatment groups (Figure 10), suggestive of a functional relationship. In addition, Pb exposure produced permanent changes in nucleus accumbens DA function in males, a region that plays a key role in mediating FI performance (Cory-Slechta et al. 1997b, 1998, 2000, 2002).

In female offspring, the impact of prenatal stress predominated in behavioral effects, with permanent elevations in FI response rates and

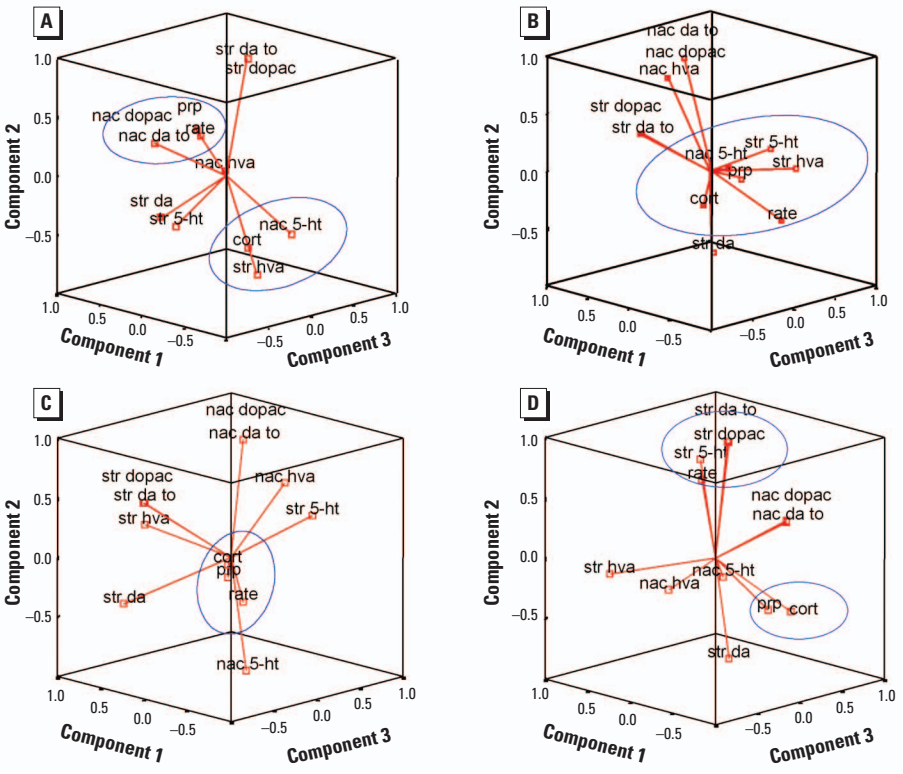

Figure 10. Factor loading plots for male offspring showing the first three components of principal component analysis carried out separately for 0/NS (*A*), S/0 (*B*), 150/NS (*C*), and 150/S (*D*), based on FI overall response rates and postreinforcement pause times from week 8, basal corticosterone levels, and adult levels of neurotransmitter, metabolites, and turnover that changed in response to Pb, stress, or Pb plus stress. Abbreviations: cort, basal corticosterone; da to, DA turnover; nac, nucleus accumbens, prp, postreinforcement pause time; rate, mean overall response rate; str, striatal. Sample sizes: NS/0 group, 7; S/0 group, 10; NS/150 group, 8; S/150 group, 8.

decreased postreinforcement pause times (Figure 9). Here, too, as with males, postreinforcement pause time and rate clustered with basal corticosterone levels in the corresponding principal component analysis, even though stress alone did not affect basal corticosterone levels in female offspring (Figure 3). Neurochemical changes, however, as measured in adulthood, were not particularly indicative of stress-alone effects. Pb exposure per se, although not associated with changes in FI performance in female offspring, did result in permanent changes in brain neurochemistry, particularly in the nucleus accumbens.

Sex-related differences in response to stress per se are well documented (Canfield et al. 2003; Faraday 2002; Shalev and Weiner 2001), with various outcomes dependent upon such factors as stressor and duration. For the impact of Pb exposure, however, sex-related differences have received surprisingly little systematic attention to date, particularly as they relate to central nervous system function. In fact, sex effects have most often been statistically controlled for in prospective cohort studies of Pb, with the exception of the Port Pirie cohort study (McMichael et al. 1988; Tong et al. 2000), which showed enhanced sensitivity of girls to the cognitive deficits associated with such exposures. One important differential change observed in this study was in basal corticosterone levels, which were elevated in response to Pb exposure alone in males but only by combined Pb plus stress in females. How sex-based differences in response to these variables may relate to the differential actions of estrogen on brain during development remains to be fully elaborated (McEwen 2001).

It is not possible to elaborate mechanisms and pathways by which observed changes in corticosterone, neurochemistry, and behavior occurred in these studies at the present. Nevertheless, important hypotheses are suggested by these findings, such as the increases in corticosterone levels induced by Pb and Pb plus stress and the intriguing link between these to FI changes in both sexes in accompanying principal component analyses. These possibilities will need to be examined more directly. It will also be important to determine how the observed pattern of effects might differ in relation to other behavioral functions differently mediated by these neurotransmitter systems, as well as in relation to even lower levels of maternal Pb exposure and to sustained Pb exposures across the lifetime.

In some cases, the nature of the resulting interactions could be perceived as a reversal of effects produced by either factor alone, that is, what might be construed as a type of beneficial effect. Caution against such interpretations must be exercised for several reasons. First, it is evident that interactions may change across time (e.g., compare day 21 vs. adult data in Table 4), and thus any interpretation based on a single time point may be misleading. We have repeatedly seen such time-related changes in Pb-induced alterations of DA function in our previous studies (McMichael et al. 1988; Pokora et al. 1996; Tong et al. 2000; Zuch et al. 1998). Furthermore, it is also evident that the nature of the observed effects varies by brain region, making it difficult to ascribe a beneficial outcome in one region but not in another, for example.

Collectively, these types of interactions have particularly significant implications for the risk assessment process. Typical risk assessment strategies use a no observed adverse effect level (NOAEL) or lowest observed adverse effect level (LOAEL) for an environmental contaminant and then include additional safety factors in defining safe levels of exposure. The findings here, however, show that interactions between Pb exposure and environmental stress are not easily predictable. This is always likely to be a significant issue when considering interactions of chemicals whose effects are not selective or specific but that may affect multiple organs or systems, and particularly if exposures to these occur in conjunction with intercurrent risk factors that also have a broad array of physiologic effects. Moreover, whether current risk assessment strategies can accommodate potentiated effects, that is, the absence of a significant effect for either risk factor alone (e.g., basal corticosterone in females in this study) and presence of a significant effect only for the combination, needs to be evaluated. Greater attention to the problems arising from interactions of risk factors must certainly be considered, given that such a scenario, in contrast to individual chemical exposures, constitutes the environmental reality.

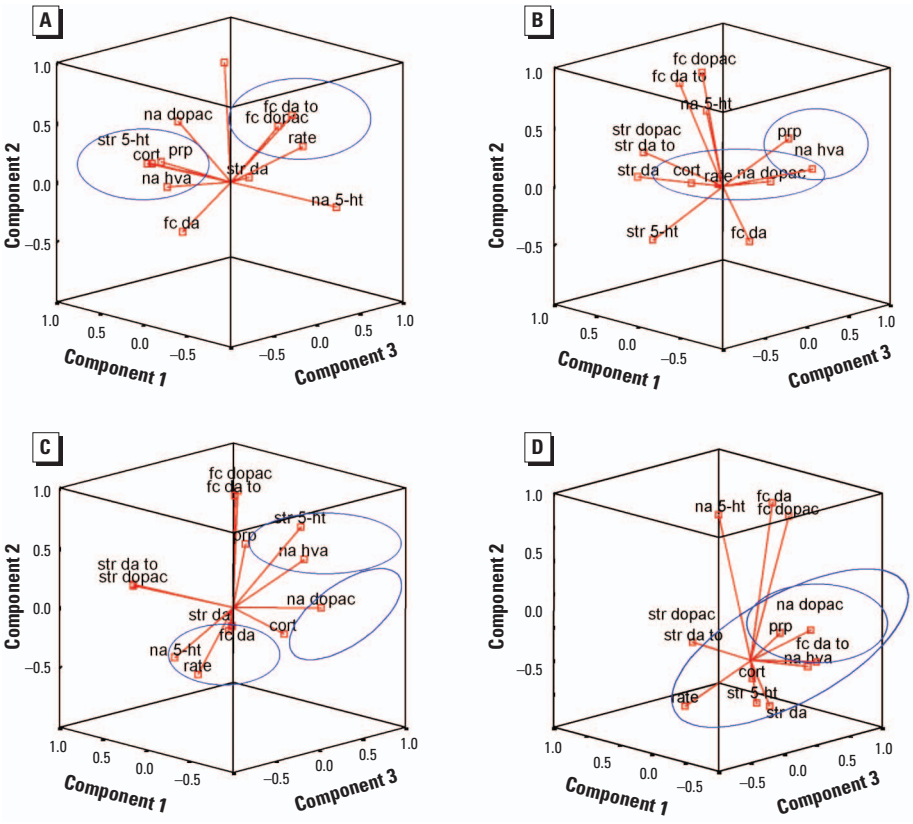

Figure 11. Factor loading plots for female offspring showing the first three components of principal component analysis carried out separately for 0/NS (*A*), S/0 (*B*), 150/NS (*C*), and 150/S (*D*), based on FI overall response rates and postreinforcement pause times from week 8, basal corticosterone levels, and adult levels of neurotransmitter, metabolites, and turnover that changed in response to Pb, stress, or Pb plus stress. Abbreviations: cort, basal corticosterone; da to, DA turnover; fc, female control; na, nucleus accumbens; prp, postreinforcement pause time; rate, mean overall response rate; str, striatal. Sample sizes: NS/0 group, 8; S/0 group, 11; NS/150 group, 7; S/150 group, 10.

Correction

Figure 8 was merged into Figure 9 in the PDF published online, therefore altering data. This has been corrected here.

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