

Brain Hemispheric Differences in the Neurochemical Effects of Lead, Prenatal Stress, and the Combination and Their Amelioration by Behavioral Experience

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Brain lateralization, critical to mediation of cognitive functions and to “multitasking,” is disrupted in conditions such as attention deficit disorder and schizophrenia. Both low-level lead (Pb) exposure and prenatal stress (PS) have been associated with mesocorticolimbic system–mediated executive-function cognitive and attention deficits. Mesocorticolimbic systems demonstrate significant laterality. Thus, altered brain lateralization could play a role in this behavioral toxicity. This study examined laterality of mesocorticolimbic monoamines (frontal cortex, nucleus accumbens, striatum, midbrain) and amino acids (frontal cortex) in male and female rats subjected to lifetime Pb exposure (0 or 50 ppm in drinking water), PS (restraint stress on gestational days 16–17), or the combination with and without repeated learning behavioral experience. Control males exhibited prominent laterality, particularly in midbrain and also in frontal cortex and striatum; females exhibited less laterality, and this was primarily striatal. Lateralized Pb ± PS induced neurotransmitter changes were assessed only in males because of limited sample sizes of Pb + PS females. In males, Pb ± PS changes occurred in left hemisphere of frontal cortex and right hemisphere of midbrain. Behavioral experience modified the laterality of Pb ± PS–induced neurotransmitter changes in a region-dependent manner. Notably, behavioral experience eliminated Pb ± PS neurotransmitter changes in males. These findings underscore the critical need to evaluate both sexes and brain hemispheres for the mechanistic understanding of sex-dependent differences in neuro- and behavioral toxicity. Furthermore, assessment of central nervous system mechanisms in the absence of behavioral experience, shown here for males, may constitute less relevant models of human health effects.

Key Words: lead; prenatal stress; laterality; dopamine; glutamate; sex.

Brain lateralization has been recognized since the nineteenth century and more recently have been highlighted in studies evaluating vocal control in the chaffinch (Nottebohm, 1970, 1971) and determining behavioral laterality in rats

(Denenberg, 1983, 1984; Denenberg *et al.*, 1980; Rosen *et al.*, 1984; Sherman *et al.*, 1980, 1983). Laterality is observed across a wide range of species, both vertebrates and invertebrates, and in multiple brain regions (Toga and Thompson, 2003; Vallortigara, 2005). Notable sex differences in asymmetries have been described, being more pronounced in males than in females (Tian *et al.*, 2011), and ascribed, at least in part, to sex hormones including testosterone (Schuepbach *et al.*, 2012; Wisniewski, 1998). These observations may relate to mechanisms of sex differences in central nervous system (CNS) function. Individual differences in laterality and which hemisphere dominates are also seen, e.g., paw preference in rodents shows an approximately 50-50 left-right distribution (Sullivan *et al.*, 2012).

Asymmetries in multiple different behavioral functions include a left hemispheric dominance for language processing (Broca, 1861) and right hemispheric dominance for visuospatial processing and spatial memory (Abrahams *et al.*, 1997; Vogel *et al.*, 2003), although the degree of lateralization for cognitive performance can be task dependent. Rodents have also been reported to exhibit behavioral laterality for complex functions, with right hemispheric dominance of spatial memory and left hippocampal bias for information transfer (Klur *et al.*, 2009; Shinohara *et al.*, 2012). The curve relating laterality to cognitive function has been described as an inverted U shape, wherein performance deteriorates at either extreme end (Hirnstein *et al.*, 2010).

Laterality has been argued to confer an evolutionary advantage, particularly for cognitive processing (Hirnstein *et al.*, 2010), minimizing conflict between the two hemispheres and permitting parallel processing or multitasking capabilities. For example, lateralization in chicks occurred with the ability to simultaneously find food and maintain vigilance against possible predators (Rogers *et al.*, 2004). Notably, altered lateralization has been associated with conditions such as schizophrenia

(Oertel-Knochel and Linden, 2011), stroke (Tecchio *et al.*, 2007), dyslexia (Leonard and Eckert, 2008), attention deficit hyperactivity disorder (Keune *et al.*, 2011; Shaw *et al.*, 2009), and autism (Lange *et al.*, 2010; Lo *et al.*, 2011).

Low-level environmental lead (Pb) exposure and prenatal stress (PS), co-occurring risk factors for low socioeconomic status communities (Scott and Nguyen, 2011), adversely influence executive functions and cognition (Brockel and Cory-Slechta, 1998; Canfield *et al.*, 2003, 2004; Cohn *et al.*, 1993; Jett *et al.*, 1997; King and Laplante, 2005; Kuhlmann *et al.*, 1997; Lanphear *et al.*, 2005; Laplante *et al.*, 2004; Lim *et al.*, 2005; Lordi *et al.*, 2000; Lui *et al.*, 2011; Sun *et al.*, 2005; Yang *et al.*, 2007) and attention (Grizenko *et al.*, 2008; Motlagh *et al.*, 2010; Nigg *et al.*, 2008, 2010) in humans and animal models. Exposures to Pb and to prenatal restraint stress in combination can actually further enhance CNS toxicity, with effects that are frequently sex dependent (Cory-Slechta *et al.*, 2010, 2012b; Froehlich *et al.*, 2007; Virgolini *et al.*, 2008b). Both Pb and PS also adversely influence the brain mesocorticolimbic systems that mediate these behaviors (Barrot *et al.*, 2000; Cory-Slechta *et al.*, 1998, 1999; Diorio *et al.*, 1993; Piazza *et al.*, 1996). Importantly, mesocorticolimbic dopamine/glutamate systems exhibit laterality in neurochemical and biochemical function (Afonso *et al.*, 1993; Belcheva *et al.*, 1990; Carlson *et al.*, 1993; Drew *et al.*, 1986; Rosen *et al.*, 1984; Sullivan, 2004; Sullivan *et al.*, 2009; Yang *et al.*, 2007).

Several questions arise from these observations. First, how widespread and sex dependent is laterality within mesocorticolimbic circuits that are critical to mediation of cognitive functions? Do the effects of Pb \pm PS differ by hemisphere in brain mesocorticolimbic systems? If so, how might these relate mechanistically to the behavioral toxicity of Pb \pm PS? Can such differences begin to explain, at a mechanistic level, the sex differences often observed in response to Pb and to PS and to the combination of these two developmental risk factors?

To begin to address these questions, this study had several aims. The first was to more fully assess the extent of lateralization and associated sex differences in mesocorticolimbic monoamine and glutamatergic neurotransmitter systems by determining levels in frontal cortex, nucleus accumbens, striatum, and midbrain. The second aim was to determine whether the neurotransmitter consequences of Pb \pm PS exhibited lateralization in mesocorticolimbic systems, and, if so, how this might relate to the effects of Pb \pm PS on cognitive behaviors in males (Cory-Slechta, 2012a). Finally, it examined the potential for a repeated learning behavioral experience to differentially influence these lateralized Pb \pm PS neurotransmitter changes.

MATERIALS AND METHODS

Dams and Pb Exposure

Three-week-old female Long Evans rats (Charles River, Germantown, NY) were randomly assigned to receive distilled deionized water drinking solutions containing 0 or 50 ppm Pb acetate and housed by same Pb-treatment condition as pairs. Pb exposure was initiated 2 months prior to breeding to ensure elevated bone Pb levels and Pb body burden at the time of conception and was continued throughout lactation, consistent with human exposure. When

females reached 3 months of age, they were paired with 3-month-old male Long Evans rats for breeding. Animals were housed in a vivarium room maintained at $22 \pm 5^\circ\text{C}$ with a 12-h light-dark cycle (lights on at 0700h). Standard rat chow diet was provided *ad libitum*. All experiments were carried out according to NIH Guidelines and were approved by the University of Rochester Medical School University Committee on Animal Resources.

Breeding and PS

At proestrus, as determined by vaginal smears, female rats were mated with males (2:1) across two estrous cycles. The presence of vaginal plugs or sperm was considered indicative of pregnancy and deemed gestational day 1 (GD1). Pregnant females in the 0 and 50 ppm Pb-treated groups were weighed and further randomly subdivided to a nonstress (NS) or PS condition. Beginning on GD1, all pregnant females were individually housed for the remainder of pregnancy and lactation.

On GD16 and 17, timed to correspond to the development of key brain regions (hypothalamic nuclei, hippocampus, striatum, frontal cortex) associated with the endpoints under study (Diaz *et al.*, 1997; Yi *et al.*, 1994), dams assigned to PS groups were weighed and subjected to a widely employed restraint stress procedure consisting of three 45-min restraint sessions (0900, 1200, and 1500h) in plastic cylindrical devices (Ward and Weisz, 1984), a protocol we have previously verified to elevate corticosterone levels and alter catecholamine levels in frontal cortex and nucleus accumbens of dams (Cory-Slechta *et al.*, 2004). NS dams were weighed and subsequently left undisturbed in their home cages. This resulted in four Pb-stress conditions with the following numbers of litters: 0-NS ($n = 12$), 0-PS ($n = 9$), 50-NS ($n = 8$), and 50-PS ($n = 8$).

Offspring Procedures

At delivery (postnatal day 1, PND1), litter size was recorded and number of pups culled to eight per litter, maintaining equal numbers of males and females wherever possible. Pups were weighed and weaned at PND21 and separated by gender, and littermates of the same gender were housed as pairs.

From weaning, pups were provided with unrestricted access to the same standard rat chow and to the same Pb drinking solutions that their dam had received. At 2 months of age, behavioral testing was initiated in a subset of pups from each treatment group using a single offspring/gender per litter ($n = 8-12/\text{group}$). A subset of additional pups from these litters were maintained as nonbehaviorally tested offspring in sample sizes of $n = 4, 5, 3$, and 4 for the 0-NS, 0-PS, 50-NS, and 50-PS groups of males, respectively, and $n = 5, 4, 3$, and 2, respectively, for females, each of which was derived from independent litters. Because sample sizes for the female 50-PS group were limited, only data for the 0-NS sex comparisons are presented; lateralization of Pb \pm PS effects and influence of behavioral testing are not presented.

With the initiation of behavioral testing at 2 months of age, all offspring (behaviorally tested and nontested) were provided with sufficient food to allow a 2–3 g weight gain per day until reaching approximately 250 g (females) or 300 g (males). At this point, caloric intake was regulated for the duration of the experiment to maintain this weight as required for food-motivated behavioral performance of the behaviorally tested subgroup. Because animals were pair housed, individual feeding was accomplished by separating the residents through the introduction of a cage divider at the time of feeding, which remained in place for approximately 90–120 min.

At the completion of behavioral testing, when all offspring were approximately 10 months of age, all animals were sacrificed and brains rapidly removed, dissected, and stored at -80°C until used for determinations of monoamines, glutamate (GLu), and its metabolite glutamine, and for gamma amino butyric acid (GABA).

Behavioral Testing on a Multiple Repeated Learning and Performance Schedule

Behavioral test sessions were carried out Mon-Fri between 1000 and 1600h in rat operant chambers (ENV-008; Med Associates, St Albans, VT) housed in sound-attenuated enclosures ventilated by a fan, equipped with a grid floor, speaker, house light, and three standard (nonretractable) response levers (L, Left; C, Center; R, Right) configured horizontally on the front panel.

Reinforcement consisted of the delivery of a 45-mg food pellet (Bioserv, Frenchtown, NJ) and behavioral contingencies, and data were controlled by SoftCtrl Cumulative Record interface and Med-PC Version IV Research Control and Data Acquisition software.

Assessment of learning relied on performance under a multiple schedule comprised of repeated learning (RL) and performance (P) components, a paradigm we have previously shown to be sensitive to postweaning Pb exposure in male rats (Cohn *et al.*, 1993), with outcomes for males and females from this study already reported (Cory-Slechta *et al.*, 2010; Cory-Slechta *et al.*, 2012a). In the RL components, the subject was required to learn a sequence of three lever press responses, with each correct sequence resulting in food reinforcement; the correct three-response sequence changed with each successive experimental session. In the P components, the subject was required to complete a sequence of three responses, but the specific sequence was constant across sessions (see below).

Assessment of behavior under the multiple schedule was preceded by lever press training (one session on fixed ratio 1 to 100 reinforcers carried out separately for each lever) followed by two- and three-response sequence RL training, as previously detailed (Cohn *et al.*, 1993). Advancement from two- to three-response sequence training occurred after 22 behavioral test sessions and from three-response sequence training to the multiple schedule after 23 behavioral test sessions. Subsequently, the multiple schedule was implemented, where each behavioral test session comprised two presentations each of the RL and P components in the order RL1, P1, RL2, P2, with each component lasting for 15 min or until 25 reinforcer deliveries, whichever occurred first. Different discriminative stimuli (lights above the three levers) were used to signal whether the RL or P component of the multiple schedule was currently in effect on the multiple schedule.

Sequences used for three-response RL training included CRL, RCL, CLR, RLC, LRC, and LCR; sequences with duplicative responses, e.g., LLR, were excluded to minimize reinforcement of perseverative responding. Based on the high accuracy levels it sustained during three-response sequence training, the sequence LCR was assigned as the correct sequence for the P components on the multiple schedule, and the remaining five sequences were used for the RL components, with each of these sequences occurring every 5th behavioral test session. Any error during the completion of the sequence in either the RL or the P components initiated a 2-s time-out and turned off the house light. Responses during the time-out extended the time-out until 2 s had elapsed without a response. Subjects were then required to begin the sequence anew. All correct responses produced a tone. Correct completion of a sequence resulted in food delivery, a tone stimulus, and the auditory click of the pellet delivery device. Performance measures included accuracy (correct responses divided by total responses), response rate (responses/time), total responses per completed sequence (total correct and incorrect responses/total completed sequences), session length (total session time), reinforcement density (total reinforcers/total responses), and total RL reinforcers (number of reinforcers earned in RL1 and RL2).

Blood Pb Determinations

Blood was collected from tail nicks in a subset of dams at three time points prior to the initiation of breeding ($n = 10$ at 3 weeks, $n = 5$ at 2 weeks, and $n = 5$ at 1 week prior to breeding) and at weaning ($n = 2$). Trunk blood was collected from offspring sacrificed at 5–6 days of age ($n = 3$ and 6, NS and PS, respectively) and from tail nicks in nonbehaviorally tested female offspring at 2.5 months of age ($n = 4$ each, NS and PS offspring) and in behaviorally tested offspring at the completion of behavioral testing at approximately 10 months of age (0-NS = 12, 0-PS = 9, 50-NS = 8, and 50-PS = 8). Blood Pb was analyzed by anodic stripping voltammetry using the Lead Care II system with a detection limit of 3.3 $\mu\text{g}/\text{dl}$. All 0-NS and 0-PS values were below detection limits. Results have been reported previously (Cory-Slechta *et al.*, 2010).

Neurochemical Determinations

Sample and Stock Preparation

After rapid decapitation, regions were dissected out and placed in 0.1N perchloric acid. Tissues were sonicated and centrifuged for 20 min at $10,000 \times g$. The supernatants were stored at -80°C until analyzed by HPLC. The pellets

were digested in 1 ml 0.1N NaOH for determination of protein concentration. Obtained values for all neurotransmitters are reported in nanogram analyte/milligram protein.

Monoamines

Levels of DA (dopamine), DOPAC (dihydroxyphenylacetic acid), HVA (homovanillic acid), NE (norepinephrine), 5-HT (serotonin), and 5-HIAA (5-hydroxyindoleacetic acid) from frontal cortex, nucleus accumbens, striatum, midbrain (ventral tegmental area [VTA] and substantia nigra), and hypothalamus were analyzed using HPLC with electrochemical detection as previously detailed (Cory-Slechta *et al.*, 2004; Virgolini *et al.*, 2008a). Commercially prepared MD-TM mobile phase (Environmental Science Associates, San Francisco, CA) was utilized. Standards of DA, DOPAC, HVA, 5-HT, and 5-HIAA were assayed at the beginning of each HPLC run. DA turnover (DA TO) was calculated as the DOPAC/DA ratio.

GLu and GABA

Levels of glutamate (GLu), glutamine (GLn), and GABA in frontal cortex were also assayed using HPLC with a method based on de Freitas Silva *et al.* (2009). GLn/GLu/GABA standards were prepared in 0.1N perchloric acid at concentrations of 6 $\mu\text{g}/\text{ml}$ GLn, 12 $\mu\text{g}/\text{ml}$ GLu, and 1.2 $\mu\text{g}/\text{ml}$ GABA. A pre-column derivatization was performed by mixing 100 μl sample or standard solution, 20 μl methanolic OPA (5 mg/ml), 75 μl borate buffer (pH 9.9), and 5 μl MPA. The sample/standard solution was vortexed and injected onto the chromatographic column at a volume of 10 μl after 1 min to allow the derivatization reaction to proceed.

The HPLC system for detection of GLu and GABA consisted of a Waters 2695 Separation Module with a 100 μl sample loop and a Waters 2475 multi- λ Fluorescence Detector. The Waters XBridge C18 3.5 μm 4.6 \times 150 mm analytical column was used for chromatographic analysis. Mobile phase consisted of 0.05M sodium acetate, tetrahydrofuran, and methanol (50:1:49, vol/vol), pH 4.0, and was filtered through 0.2 μm nylon filter prior to use. Chromatographic analyses were performed at $25 \pm 2^\circ\text{C}$. Analytes were isocratically eluted over a 22-min run time at a flow rate of 0.8 ml/min. Excitation and emission wavelengths were set at 337 and 454 nm, respectively. GLn/GLu/GABA were identified by their retention times (approximately 4.5, 7.6, 18.6 min, respectively) as determined by standard injections. Standards for GLn, GLu, and GABA were assayed at the beginning of each HPLC run. GLu turnover was defined by the GLn/GLu ratio previously shown to be sensitive to be altered both in schizophrenia and in epilepsy (Hashimoto *et al.*, 2005; Petroff *et al.*, 2002).

Statistical Analysis

Blood Pb Values

Because all 0-NS and 0-PS group blood Pb levels were below detection limits, only values from 50-NS and 50-PS groups were analyzed. For dams, these analyses relied on ANOVAs with time point (three time points prebreeding and weaning) as a between-groups factor. Blood Pb values of offspring were analyzed using ANOVAs with stress (NS, PS) and time points as between-groups factors. Repeated measures analyses of variance (RMANOVAs) were not used for these analyses, since the same pool of rats was not sampled at each time point. *Post hoc* tests were carried out using Fisher's protected least significant difference tests as appropriate. These blood Pb values have been reported previously (Cory-Slechta *et al.*, 2010; Cory-Slechta *et al.*, 2012a).

Neurotransmitter Changes

Brain laterality under normal conditions. Given the complexity of the experimental design and the sample sizes, evaluation in the 0-NS only nonbehaviorally trained groups was considered definitive confirmation of mesocorticolimbic laterality under conventional conditions. Thus, for assessment of brain laterality under normal (control) conditions, RMANOVAs were carried out using hemisphere (right or left) as a within-group factor and sex as a between-group factor. These were followed appropriately by *post hoc* tests as determined by main effects or interactions.

Hemispheric differences in the effects of Pb, stress, or the combination on neurotransmitter levels. To determine whether Pb \pm PS effects differed by hemisphere in males, RMANOVAs were first carried out using Pb and PS as between-group factors and hemisphere (left or right) as a within-group factor in nonbehaviorally trained rats. Analyses were carried out separately for each neurotransmitter in each brain region. For those regions in which statistical interactions were confirmed, subsequent ANOVAs with Pb and PS as between-group factors were carried out for each hemisphere.

Effects of behavioral testing on laterality and associated Pb \pm PS neurotransmitter changes. To evaluate the extent to which behavioral testing modified Pb \pm PS-associated neurotransmitter changes in males, ANOVAs were carried out using Pb, PS, and behavioral testing condition (behaviorally tested or nonbehaviorally tested) as between-group factors. These analyses were carried out separately for each neurotransmitter in each brain region. For those regions in which statistical interactions were identified, subsequent ANOVAs as appropriate with Pb and PS as between-group factors were carried out for each behavioral testing condition.

Evaluation of the complete profile of neurotransmitter changes across regions necessarily required multiple statistical tests. For that reason, only findings involving main effects or statistical interactions were subsequently pursued statistically. Further, because of the large number of comparisons involved in these analyses, the focus was on consistency of patterns of changes. *p* Values were defined as significant at ≤ 0.05 , and given the somewhat restricted sample sizes, near significant effects are also noted where potentially relevant.

RESULTS

Blood Pb and Dam and Pup Outcomes

Blood Pb (PbBs) from dams and male and female offspring from these studies have been reported previously (Cory-Slechta *et al.*, 2010; Cory-Slechta *et al.*, 2012a). Briefly, PbB levels of dams measured at three time points prior to breeding averaged 5–7 $\mu\text{g}/\text{dl}$ and increased to 13 $\mu\text{g}/\text{dl}$ at weaning. Offspring exhibited PbBs of 10–13 $\mu\text{g}/\text{dl}$ at PND5–6; these declined to 7 $\mu\text{g}/\text{dl}$ at 2.5 months and increased at 10 months to 10 $\mu\text{g}/\text{dl}$. Neither litter size (group mean values ranging from 14.25 to 15.63 pups) nor litter weight (group mean values ranging from 82.13 to 94.00 g) was significantly influenced by Pb, PS, or the combination.

Brain Laterality Differs by Brain Region and Sex

To determine conditions of hemispheric lateralization under conventional conditions, statistical analyses were carried out using only animals from the control (0-NS) nonbehaviorally tested groups. Monoamine neurotransmitters that differed by sex and/or hemisphere in those analyses are shown in Figure 1 by brain region. Increased frontal cortex DA, reduced DA TO, and increased 5-HIAA in right hemisphere (DA: $p = 0.013$; DA TO: $p = 0.01$; HIAA: $p = 0.008$) were found in both sexes. Modest increases in right hemisphere striatal DA TO ($p = 0.006$) and in right hemispheric DOPAC ($p = 0.012$) and DA TO in midbrain ($p = 0.027$) were also seen in both sexes.

Sex-dependent differences in neurotransmitters were found primarily in striatum, where females showed lower levels of DA ($p = 0.006$), DOPAC ($p = 0.006$), and HVA ($p = 0.024$) than males.

Sex by hemisphere interactions were most prevalent in midbrain and were also seen with striatal 5-HT and HIAA. Notably,

the sex by hemisphere differences in midbrain were due to the absence of hemispheric laterality in females, in contrast to significantly higher levels of DA ($p = 0.004$), HVA ($p = 0.005$), NE ($p = 0.007$), 5-HT ($p = 0.006$), and 5-HIAA ($p = 0.006$) in right hemisphere in males. For striatal 5-HT ($p = 0.014$) and 5-HIAA ($p = 0.032$), similar interactions were noted, i.e., absence of laterality in females but greater left hemispheric levels in males.

Sex by hemisphere interactions also were seen for all three amino acids assayed in frontal cortex (Fig. 1), with higher left hemispheric levels of GLn, GLu, and GABA in males, whereas no laterality was evident in females ($p = 0.005$, 0.033, and 0.009, respectively). GLu turnover showed no differences.

Effects of Pb \pm PS Can Differ by Hemisphere

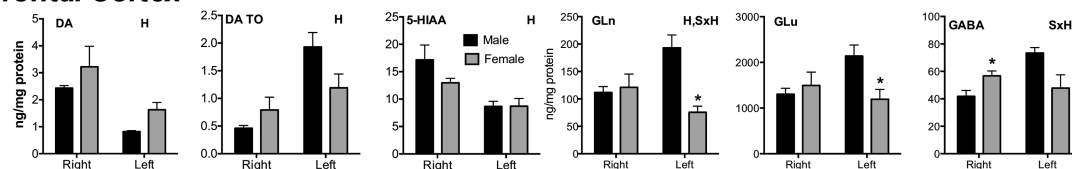
Monoamines. Significant laterality-associated differences in Pb \pm PS effects on monoamine neurotransmitter levels (based on Pb \times PS \times hemisphere, Pb \times hemisphere, or PS \times hemisphere interactions in RMANOVAs) were found only in frontal cortex and midbrain in nonbehaviorally tested males (Fig. 2). In frontal cortex, Pb \pm PS-induced changes were restricted to the left hemisphere. For frontal cortex, Pb + PS selectively increased DA TO ($p = 0.047$), and, correspondingly, marginally reduced DA ($p = 0.063$) in left hemisphere (42%; $p = 0.01$). Pb + PS also selectively reduced left hemisphere NE (21%, $p = 0.014$). No effects of Pb \pm PS were found in right hemisphere.

In contrast, Pb \pm PS interactions in midbrain were seen only in right hemisphere in nonbehaviorally tested males (DOPAC: $p = 0.047$; HVA: $p = 0.007$; NE: $p = 0.038$; 5-HT: $p = 0.045$, and HIAA: $p = 0.014$), where the general pattern consisted of Pb or Pb + PS selectively reducing monoamine levels by 12–88% (DOPAC: $p = 0.049$, HVA: $p = 0.0003$ and 0.006 for Pb and PS, respectively; NE: $p = 0.014$; and HIAA: $p = 0.005$).

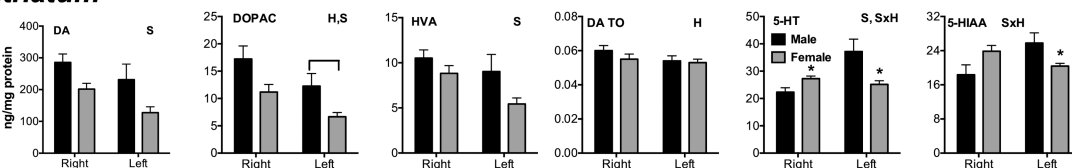
Amino acids. Significant interactions of Pb \pm PS with hemisphere were found for all three amino acids in nonbehaviorally tested males (Fig. 2; $p = 0.038$, 0.017, and 0.035 for GLn, GLu, and GABA, respectively). Although no Pb \pm PS-related effects on GLn were found in *post hoc* ANOVAs in either hemisphere, Pb significantly increased in right hemisphere GLu by 43–60% ($p = 0.021$), whereas left hemisphere levels were not significant. Left hemisphere GABA levels were significantly reduced by Pb \pm PS ($p = 0.017$) by 27–43%, whereas no changes were found in right hemisphere. GLu turnover was not differentially altered.

Individual differences in magnitude and dominant hemisphere. As has previously been reported, individual differences in laterality, including the extent to which it occurs and the dominant hemisphere, were seen in nonbehaviorally tested males (Fig. 3). Of the 4 0-NS males, one showed very little laterality, and for all four subjects, right hemisphere showed higher levels of neurotransmitter. Albeit based on somewhat limited

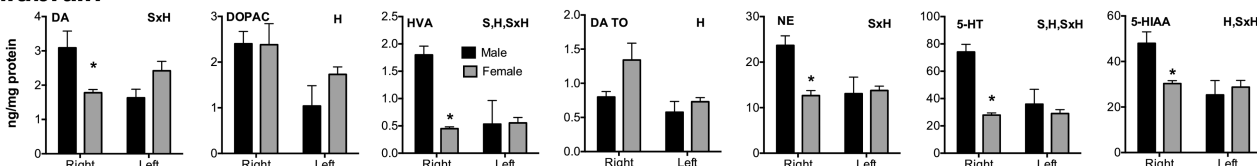
Frontal Cortex



Striatum



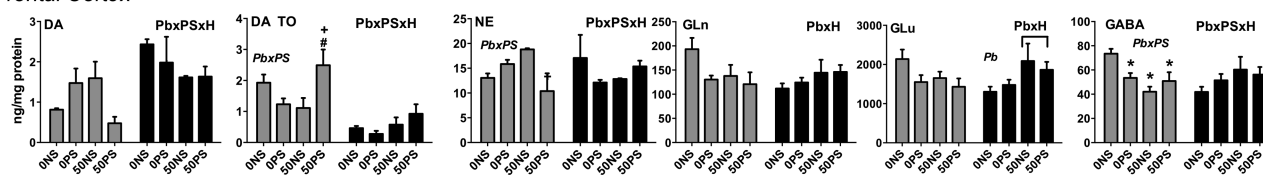
Midbrain



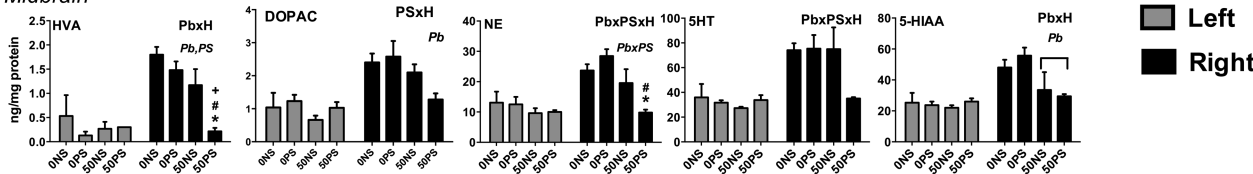
Hemisphere

FIG. 1. Group mean \pm SE neurotransmitter levels (ng/mg protein) as identified for control (0-NS only) nonbehaviorally tested males and females in right versus left hemisphere in frontal cortex, striatum, and midbrain. Only those neurotransmitters within a region that exhibited statistically significant differences are shown. H, main effect of hemisphere; S, main effect of sex; S \times H, sex by hemisphere interaction. * differs significantly from corresponding other sex outcome.

Frontal Cortex



Midbrain



Treatment Group

FIG. 2. Group mean \pm SE neurotransmitter levels (ng/mg protein) of nonbehaviorally tested males as identified for treatment groups in right versus left hemisphere in frontal cortex and midbrain. Only those neurotransmitters within a region that exhibited statistically significant differences are shown. H, main effect of hemisphere; Pb, main effect of Pb; PS, main effect of PS; Pb \times H, Pb by hemisphere interaction; PS \times H, PS by hemisphere interaction; Pb \times PS \times H, Pb by PS by hemisphere interaction in RMANOVAs comparing hemispheres. Pb \times PS, Pb by PS interaction in hemisphere-specific ANOVAs. * differs significantly from corresponding 0-NS group; + differs significantly from corresponding 0-PS group; # differs from corresponding 50-NS group.

sample sizes, it can also be seen that while right hemisphere remained dominant for midbrain, the magnitude of laterality tended to be diminished with Pb and Pb + PS. In fact, almost no laterality is evident for midbrain NE in the Pb + PS group.

Behavioral Testing Influences Pb \pm PS-Induced Neurotransmitter Changes in a Hemispheric Manner

Monoamines. In males, behavioral testing differentially influenced absolute neurotransmitter levels (e.g., main effects of behavioral testing condition [BTC]) in virtually all brain regions

examined. Interactive effects of Pb \pm PS with behavioral testing were most dramatic in frontal cortex and midbrain and were also seen in striatum, and almost invariably consisted of the absence of Pb \pm PS effects in behaviorally tested males relative to nonbehaviorally tested male littermates, as shown in Figure 4.

Frontal cortex HVA and DA TO levels were increased particularly by Pb + PS (471 and 201%, and $p = 0.031$ and 0.05 , respectively) and DOPAC levels reduced by PS alone (by 35%; $p = 0.049$) with corresponding but nonsignificant reductions in DA in nonbehaviorally tested offspring,

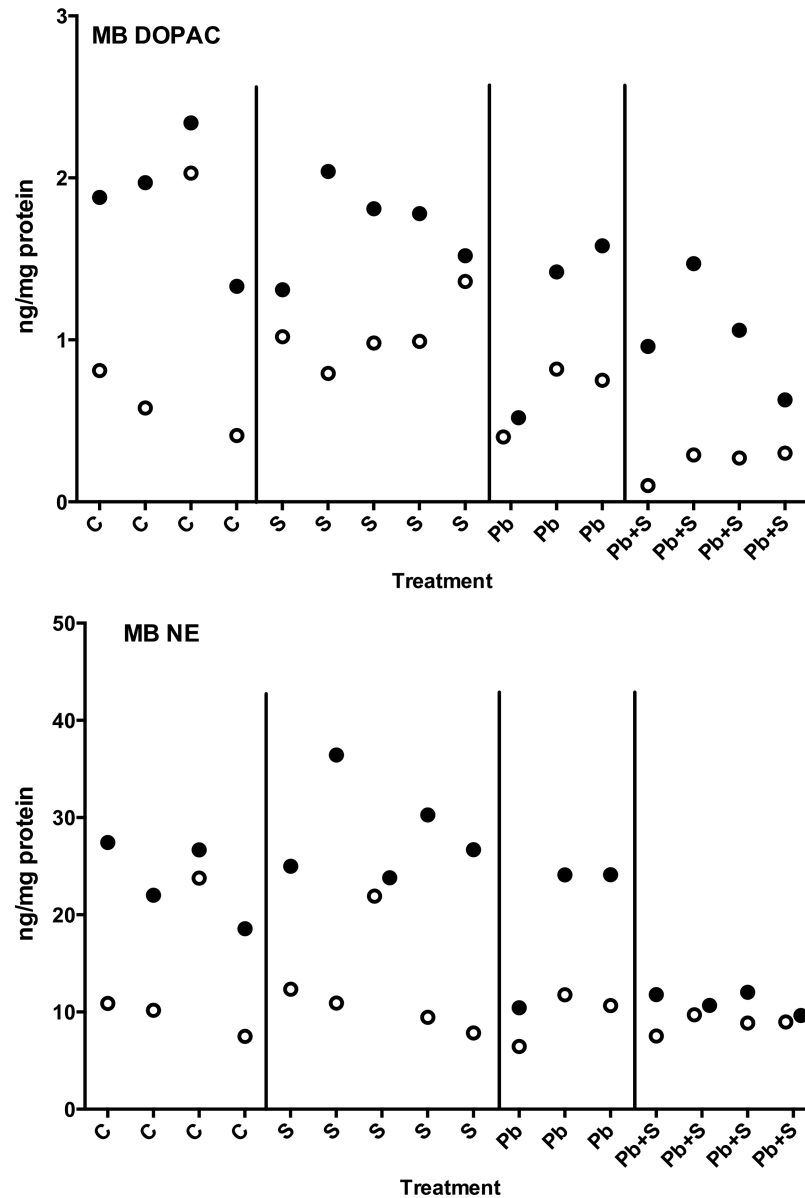


FIG. 3. Levels (ng/mg protein) of midbrain DOPAC (top) and NE (bottom) of individual male nonbehaviorally tested subjects from treatment groups as identified. Closed circles represent values from right hemisphere and open circles from left hemisphere.

whereas no Pb \pm PS effects were seen in behaviorally tested males. Pb reduced striatal DA levels by 25–56% ($p = 0.011$), whereas DA TO levels increased by 58–83% ($p = 0.001$) in nonbehaviorally tested males, but no corresponding effects were found in behaviorally tested males. In midbrain, Pb, and in some cases particularly Pb + PS, markedly reduced midbrain DOPAC, HVA, NE, and 5-HIAA levels ($p = 0.042$, 0.027 , $p < 0.0001$, and $p < 0.0001$, respectively) by 12–88%, whereas the only effect in behaviorally tested littermates consisted of a modest PS-based increase in 5-HIAA ($p = 0.022$).

Data from left hemisphere nucleus accumbens in behaviorally tested and nonbehaviorally tested males indicate that the

differential influence of behavioral testing on Pb \pm PS-induced neurotransmitter levels is not restricted to the right hemisphere (Fig. 5). In addition to BTC-associated differences in absolute neurotransmitter levels, differential effects of Pb \pm PS were found for DA ($p = 0.011$) and HVA ($p = 0.029$). PS increased DA levels by 53–61% in nonbehaviorally tested males, whereas a modest increase in the 50-PS group relative to 0-PS was found in behaviorally tested males. PS also increased HVA levels by 45–60% in nonbehaviorally tested males, whereas no HVA changes were found in behaviorally tested males.

Amino acids. Differential effects of Pb \pm PS on frontal cortex amino acids in relation to behavioral testing were largely

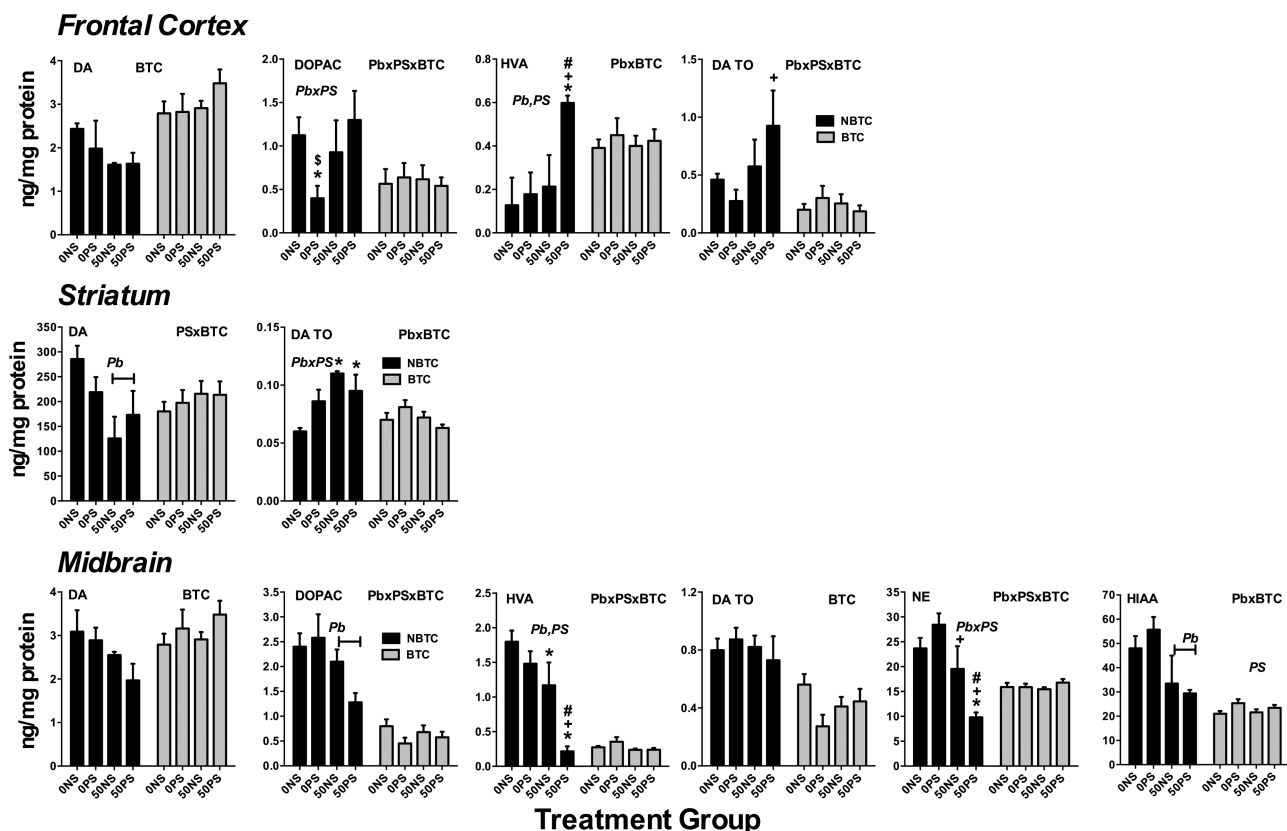


FIG. 4. Group mean \pm SE neurotransmitter levels (ng/mg protein) of males as identified for treatment groups in right hemisphere of behaviorally tested (BTC) versus nonbehaviorally tested offspring (NBTC) in frontal cortex, striatum, and midbrain. Only those neurotransmitters within a region that exhibited statistically significant interactions are shown. BTC, main effect of behavioral testing condition; Pb, main effect of Pb; PS, main effect of PS; Pb \times BTC, Pb by behavioral testing interaction; PS \times BTC, PS by behavioral testing condition interaction; Pb \times PS \times BTC, Pb by PS by behavioral testing interaction in ANOVAs comparing behavioral testing effect. Pb \times PS, Pb by PS interaction in hemisphere-specific ANOVAs. *differs significantly from corresponding 0-NS control; + differs significantly from corresponding 0-PS group; # differs from corresponding 50-NS group; \$ differs from 50-PS group.

restricted to differences in absolute neurotransmitter levels in males (data not shown).

DISCUSSION

This study demonstrated that under conventional conditions, brain lateralization is both sex and region dependent, being more prominent in males particularly in midbrain. Additionally, the effects of Pb \pm PS, examined in males, can differ significantly by hemisphere in mesocorticolimbic brain regions, with profiles of changes that differ notably by region. Further, behavioral experience, also examined only in males and in this case consisting of repeated learning experience, appears to attenuate the effects of Pb \pm PS.

Under Conventional Conditions, Hemispheric Laterality of Neurotransmitters is Found Across Mesocorticolimbic Regions in Males But to a Lesser Extent in Females

Consistent with prior studies, a broad laterality of monoamine/Glu neurotransmitters was seen in the mesocorticolimbic

system (Belcheva *et al.*, 1990; Capper-Loup *et al.*, 2009; Carlson *et al.*, 1993; Rodriguez *et al.*, 1990; Rosen *et al.*, 1984; Sullivan and Dufresne, 2006) and was more prominent in males (Berrebi *et al.*, 1988; Sullivan *et al.*, 2009; Toga and Thompson, 2003; Wisniewski, 1998). Comparisons to profiles of laterality reported in prior studies, however, are difficult given the use of mixed or single sexes or identification of regions as high versus low rather than by side (Rodriguez *et al.*, 1990; Rosen *et al.*, 1984).

Of note, midbrain monoamine laterality was striking in males, characterized by increased levels and turnover in the right hemisphere, but absent in females. Similarly, frontal cortex amino acids exhibited laterality only in males. Although sex differences in absolute neurotransmitter levels have been reported for both substantia nigra and VTA (Rodriguez *et al.*, 1990), this appears to be the first report of major sex-related difference in midbrain laterality. Its consistency in males suggests its contribution to laterality in projection regions, including frontal cortex, striatum, and nucleus accumbens. Its absence in females, even with evidence of frontal cortex monoaminergic laterality (albeit of lesser magnitude than in males), argues that other factors must contribute to cortical

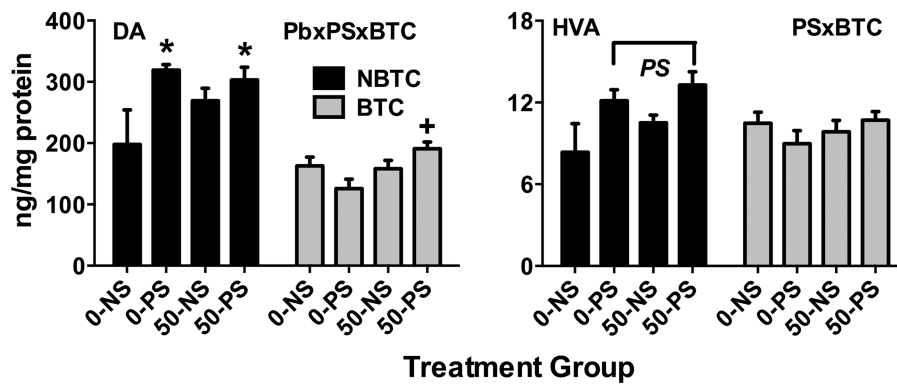


FIG. 5. Group mean \pm SE neurotransmitter levels (ng/mg protein) of males as identified for treatment groups in left hemisphere of behaviorally tested (BTC) versus nonbehaviorally tested offspring (NBTC) in nucleus accumbens. Only those neurotransmitters within this region that exhibited statistically significant interaction are shown. BTC, main effect of behavioral testing condition; PS \times BTC, PS by behavioral testing condition interaction; Pb \times PS \times BTC, Pb by PS by behavioral testing interaction in ANOVAs comparing behavioral testing effect. PS, main effect of PS; Pb \times PS, Pb by PS interaction in hemisphere-specific ANOVAs. * differs significantly from corresponding 0-NS control; + differs significantly from corresponding 0-PS group.

lateralization, e.g., sex hormones, which have been shown to influence laterality (Geschwind and Galaburda, 1985; Hausmann and Güntürkün, 2000; Toga and Thompson, 2003; Wisniewski, 1998).

The pronounced sex difference in midbrain laterality may have behavioral and disease and disorder-related mechanistic implications. Midbrain is the site of DA cell bodies of both the nigrostriatal and mesocorticolimbic DA tracts. Differences in laterality may assist in explaining sex-related differences in behaviors mediated by these systems that can include spatial working and memory and motor activity (Simpson and Kelly, 2012) and diseases and disorders associated with these DA tracts that show sex-related differences, e.g., Parkinson's disease and attention deficit disorder.

Neurochemical Changes Produced by Pb \pm PS Can Differ Significantly by Hemisphere in Mesocorticolimbic Regions

The potential for differential effects of an environmental neurotoxicant by brain hemisphere does not appear to have been previously assessed. Given limited sample sizes of Pb + PS-treated females, examination of the hemispheric effects of Pb \pm PS on brain neurotransmitter levels was carried out only in males. Neurotransmitter changes produced by Pb \pm PS in males differed by hemisphere in a brain region-dependent manner. Pb \pm PS effects in males in left hemisphere were found in frontal cortex but were prominent in right hemisphere of midbrain. Frontal cortex Pb \pm PS-related changes in amino acids were less consistent, with Pb-induced increases in Glu in right hemisphere and both PS- and Pb-associated decreases in GABA in left hemisphere (Fig. 2). Importantly, there was also suggestive evidence that these hemispheric-dependent Pb effects could be exacerbated by PS although not necessarily framed in Pb \times PS interactions, as noted for left frontal cortex DA turnover and particularly for midbrain (HVA, DOPAC, NE, and 5-HT).

Considered collectively, the highly consistent effects of Pb in right midbrain suggest it may be a primary mechanism of mesocorticolimbic system disruption by Pb (Cohn and Cory-Slechta, 1993, 1994a,b; Cory-Slechta, 1995; Cory-Slechta *et al.*, 1996, 1997; Pokora *et al.*, 1996). Further, it may contribute to the wide range of effects of Pb on CNS function. Midbrain dopaminergic neurons project to multiple regions, including frontal cortex, nucleus accumbens, striatum, hypothalamus, and hippocampus (Gasbarri *et al.*, 1991; Oades and Halliday, 1987; Sesack and Grace, 2010). Additionally, midbrain also receives afferent projections from multiple regions including areas of prefrontal cortex, nucleus accumbens, hypothalamus, dorsal raphe, and other catecholaminergic nuclei. Many of these regions, and particularly their network/circuitry functioning, are critical to mediation of cognitive functions and to mediation of appetitive behavior and of reward (Sesack and Grace, 2010).

Although future studies will be required to define the relative importance of VTA compared with substantia nigra to the lateralized effects of Pb \pm PS effects in midbrain, the potential analogy of lateralized midbrain dopaminergic dysfunction to the consequences of unilateral lesions of VTA is intriguing in that augmented function of an intact hemisphere can be found after unilateral injury, suggesting a compensatory mechanism. Specifically, unilateral lesions of VTA, and thus presumably reduced dopaminergic function, in rat have been shown to enhance contralateral VTA-stimulated feeding and exploration and locomotion (Maliszewska-Scislo and Trojniar, 1999; Trojniar and Klejbor, 1999). Several mechanisms have been suggested to account for such findings. One postulates increased DA levels in the terminal dopaminergic structures of the other hemisphere (Maliszewska-Scislo and Trojniar, 1999); in that regard, Pb \pm PS-induced increases in left hemisphere frontal cortex DA turnover were observed, but differential Pb \pm PS-induced neurotransmitter changes in nucleus accumbens were not notable. A second potential mechanism involves the VTA

lesion sensitizing the mesolimbic system by suppressing inhibitory afferents to the contralateral VTA (Majkutewicz *et al.*, 2010); data from the current study do not allow an assessment of this hypothesis.

Numerous other studies already document that brain lateralities extend from morphological to biochemical outcomes, including such diverse measures of CNS function as D2 receptor density (Drew *et al.*, 1986), hippocampal gene expression (Klur *et al.*, 2009), and cell proliferation (Czéh *et al.*, 2008), indicating that laterality is characteristic of brain functioning. These findings raise critical mechanistic questions. For example, which hemisphere should be used to mechanistically relate the effects of Pb ± PS to behavioral function? Should it differ by region? Or would it rely on an understanding of the consequences as integrated across the two hemispheres? Clearly, assessment in only one hemisphere may be misrepresentative, i.e., assessment of only right frontal cortex or left midbrain would have led to the conclusion of no Pb ± PS effects.

From a methodologic point of view, few studies define which hemisphere was used for neurochemical or biochemical assessments, or whether it was the same across all animals. Also clear from these findings is that random use of right or left hemisphere may result in negative findings if effects differ by hemisphere. Hemispheric differences are unlikely to be restricted to Pb ± PS but will likely be characteristic of other developmental neurotoxicants as well. Indeed, prenatal alcohol exposure demonstrates lateralized effects (Zimmerberg and Reuter, 1989).

Behavioral Experience Appears to Reverse

Hemisphere-Dependent Effects of Pb ± PS in Males

The findings also show that neurotransmitter levels and metabolism can differ significantly in relation to behavioral experience in a region-dependent context. Here, behavioral experience appeared to mitigate the effects of Pb ± PS in male offspring. Whereas numerous Pb ± PS-induced neurotransmitter changes were seen in males lacking behavioral experience, only a single PS-induced increase in midbrain HIAA was seen in males with behavioral experience.

It could be presumed that behavioral experience on the multiple schedule of repeated learning and performance in this study functioned much like an “environmental enrichment.” Environmental enrichment paradigms have been reported to attenuate effects of Pb and of PS (Fox *et al.*, 2006; Guilarte *et al.*, 2003; Schneider *et al.*, 2001). As has been shown in other studies, however, the impact of environmental enrichment depends upon numerous different factors, outcome can often differ by gender, and “enrichment,” depending upon how formulated, can actually worsen outcome (Simpson and Kelly, 2011).

Importantly, the fact that Pb ± PS can produce neurochemical changes that differ by hemisphere and that behavioral testing can alter the neurochemical consequences of Pb ± PS in a hemispheric capacity, certainly complicates the assessment of neurochemical mechanisms of behavioral

toxicity. From the simplest point of view, one might surmise that the *enhanced learning* seen with Pb ± PS in behaviorally tested males (Cory-Slechta *et al.*, 2012a) compared with the *enhanced impairments in learning accuracy* arising from Pb + PS in behaviorally tested females (Cory-Slechta *et al.*, 2010) reflected in part the differential “enrichment” capacities of the behavioral testing, wherein Pb ± PS-associated neurochemical changes were virtually eliminated in males but without apparent effects in females. Increased learning accuracy in males appeared to have been the result of increased response rates with a resulting increase in reinforcement density (Cory-Slechta *et al.*, 2012a). Understanding the mechanisms of increased response rates in the males may require comparisons of the pattern of associated neurochemical changes in nonbehaviorally tested males, such as altered levels of DA and turnover seen in frontal cortex, striatum, and midbrain, compared with those in behaviorally tested littermates because it is clear that behavioral testing *per se* can change brain neurochemistry (Cory-Slechta *et al.*, 2009) and can also alter the consequences of Pb ± PS (Cory-Slechta *et al.*, 2012b). It will ultimately require an understanding of how behavioral performance(s) interact with compensatory neurotransmitter changes as seen in nonbehaviorally tested males.

In summary, the current findings demonstrate that Pb ± PS can induce neurochemical changes that are hemisphere dependent. Additionally, behavioral testing, whether fixed interval schedule controlled performance (Cory-Slechta *et al.*, 2009) or repeated learning, as shown here, can alter the neurochemical consequences of Pb ± PS in a hemisphere-dependent manner. These observations have many implications. To the best of our knowledge, this is the first study to demonstrate hemispheric laterality of the effects of an environmental developmental neurotoxicant, here Pb and/or PS. However, given that laterality extends to multiple aspects of CNS structure and function (Czéh *et al.*, 2008; Drew *et al.*, 1986; Klur *et al.*, 2009; Toga and Thompson, 2003) and that prior studies have indicated that prenatal ethanol can alter laterality (Zimmerberg and Reuter, 1989), it seems highly likely that other environmental neurotoxicants might also differ in their effects by hemisphere. Based on these findings, it is possible that assessment of behavioral or biochemical outcomes only in behaviorally tested offspring may be misinformative and that ultimately information from both hemispheres, as well as from behaviorally tested and nonbehaviorally tested brains, is required for such a purpose. It is also evident that the hemisphere used in analyses should be explicitly identified. These findings also underscore the imperative need to examine neurochemical and likely biochemical CNS effects in both sexes, given the broad sex dependence of laterality under conventional conditions seen here. Moreover, based on the sex differences, it would seem that different therapeutic interventions, whether behavioral or pharmacological, might be required for Pb ± PS-induced CNS toxicity in males versus females.

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REFERENCES

- Abrahams, S., Pickering, A., Polkey, C. E., and Morris, R. G. (1997). Spatial memory deficits in patients with unilateral damage to the right hippocampal formation. *Neuropsychologia* **35**, 11–24.
- Afonso, D., Santana, C., and Rodriguez, M. (1993). Neonatal lateralization of behavior and brain dopaminergic asymmetry. *Brain Res. Bull.* **32**, 11–16.
- Barrot, M., Marinelli, M., Abrous, D. N., Rougé-Pont, F., Le Moal, M., and Piazza, P. V. (2000). The dopaminergic hyper-responsiveness of the shell of the nucleus accumbens is hormone-dependent. *Eur. J. Neurosci.* **12**, 973–979.
- Belcheva, I., Bryer, J. B., Starkstein, S. E., Honig, M., Moran, T. H., and Robinson, R. G. (1990). Hemispheric asymmetry in behavioral response to D1 and D2 receptor agonists in the nucleus accumbens. *Brain Res.* **533**, 286–291.
- Berberi, A. S., Fitch, R. H., Ralphe, D. L., Denenberg, J. O., Friedrich, V. L., Jr, and Denenberg, V. H. (1988). Corpus callosum: Region-specific effects of sex, early experience and age. *Brain Res.* **438**, 216–224.
- Broca, P. (1861). Remarques sur le siege de la faculte du langage articule suivies d'une observation d'aphemie (perte de la parole). *Bull. Soc. Anthropol.* **6**, 330–357.
- Brockel, B. J., and Cory-Slechta, D. A. (1998). Lead, attention, and impulsive behavior: Changes in a fixed-ratio waiting-for-reward paradigm. *Pharmacol. Biochem. Behav.* **60**, 545–552.
- Canfield, R. L., Gendle, M. H., and Cory-Slechta, D. A. (2004). Impaired neuropsychological functioning in lead-exposed children. *Dev. Neuropsychol.* **26**, 513–540.
- Canfield, R. L., Henderson, C. R., Jr, Cory-Slechta, D. A., Cox, C., Jusko, T. A., and Lanphear, B. P. (2003). Intellectual impairment in children with blood lead concentrations below 10 microg per deciliter. *N. Engl. J. Med.* **348**, 1517–1526.
- Capper-Loup, C., Rebell, D., and Kaelin-Lang, A. (2009). Hemispheric lateralization of the corticostriatal glutamatergic system in the rat. *J. Neural Transm.* **116**, 1053–1057.
- Carlson, J. N., Fitzgerald, L. W., Keller, R. W., Jr, and Glick, S. D. (1993). Lateralized changes in prefrontal cortical dopamine activity induced by controllable and uncontrollable stress in the rat. *Brain Res.* **630**, 178–187.
- Cohn, J., and Cory-Slechta, D. A. (1993). Subsensitivity of lead-exposed rats to the accuracy-impairing and rate-altering effects of MK-801 on a multiple schedule of repeated learning and performance. *Brain Res.* **600**, 208–218.
- Cohn, J., and Cory-Slechta, D. A. (1994a). Assessment of the role of dopaminergic systems in lead-induced learning impairments using a repeated acquisition and performance baseline. *Neurotoxicology* **15**, 913–926.
- Cohn, J., and Cory-Slechta, D. A. (1994b). Lead exposure potentiates the effects of NMDA on repeated learning. *Neurotoxicol. Teratol.* **16**, 455–465.
- Cohn, J., Cox, C., and Cory-Slechta, D. A. (1993). The effects of lead exposure on learning in a multiple repeated acquisition and performance schedule. *Neurotoxicology* **14**, 329–346.
- Cory-Slechta, D. A. (1995). Relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic, and glutamatergic neurotransmitter system functions. *Annu. Rev. Pharmacol. Toxicol.* **35**, 391–415.
- Cory-Slechta, D. A., Merchant-Borna, K., Allen, J., Liu, S., Weston, D., and Conrad, K. (2012a). Enhanced stimulus sequence-dependent repeated learning in male offspring after prenatal stress alone or in conjunction with lead exposure. *Neurotoxicology* **33**, 1188–1202.
- Cory-Slechta, D. A., Merchant-Borna, K., Allen, J. L., Liu, S., Weston, D., and Conrad, K. (2012b). Variations in the nature of behavioral experience can differentially alter the consequences of developmental exposures to lead, prenatal stress, and the combination. *Toxicol. Sci.* **131**, 194–205.
- Cory-Slechta, D. A., O'Mara, D. J., and Brockel, B. J. (1998). Nucleus accumbens dopaminergic medication of fixed interval schedule-controlled behavior and its modulation by low-level lead exposure. *J. Pharmacol. Exp. Ther.* **286**, 794–805.
- Cory-Slechta, D. A., O'Mara, D. J., and Brockel, B. J. (1999). Learning versus performance impairments following regional administration of MK-801 into nucleus accumbens and dorsomedial striatum. *Behav. Brain Res.* **102**, 181–194.
- Cory-Slechta, D. A., Pazmino, R., and Bare, C. (1997). The critical role of nucleus accumbens dopamine systems in the mediation of fixed interval schedule-controlled operant behavior. *Brain Res.* **764**, 253–256.
- Cory-Slechta, D. A., Pokora, M. J., and Preston, R. A. (1996). The effects of dopamine agonists on fixed interval schedule-controlled behavior are selectively altered by low-level lead exposure. *Neurotoxicol. Teratol.* **18**, 565–575.
- Cory-Slechta, D. A., Stern, S., Weston, D., Allen, J. L., and Liu, S. (2010). Enhanced learning deficits in female rats following lifetime pb exposure combined with prenatal stress. *Toxicol. Sci.* **117**, 427–438.
- Cory-Slechta, D. A., Virgolini, M. B., Liu, S., and Weston, D. (2012c). Enhanced stimulus sequence-dependent repeated learning in male offspring after prenatal stress alone or in conjunction with lead exposure. *Neurotoxicology*.
- Cory-Slechta, D. A., Virgolini, M. B., Liu, S., and Weston, D. (2012d). Enhanced stimulus sequence-dependent repeated learning in male offspring after prenatal stress alone or in conjunction with lead exposure. *Neurotoxicology* **33**, 1188–1202.
- Cory-Slechta, D. A., Virgolini, M. B., Rossi-George, A., Weston, D., and Thiruchelvam, M. (2009). Experimental manipulations blunt time-induced changes in brain monoamine levels and completely reverse stress, but not Pb+-stress-related modifications to these trajectories. *Behav. Brain Res.* **205**, 76–87.
- Cory-Slechta, D. A., Virgolini, M. B., Thiruchelvam, M., Weston, D. D., and Bauter, M. R. (2004). Maternal stress modulates the effects of developmental lead exposure. *Environ. Health Perspect.* **112**, 717–730.
- Czéh, B., Perez-Cruz, C., Fuchs, E., and Flügge, G. (2008). Chronic stress-induced cellular changes in the medial prefrontal cortex and their potential clinical implications: Does hemisphere location matter? *Behav. Brain Res.* **190**, 1–13.
- de Freitas Silva, D. M., Ferraz, V. P., and Ribeiro, A. M. (2009). Improved high-performance liquid chromatographic method for GABA and glutamate determination in regions of the rodent brain. *J. Neurosci. Methods* **177**, 289–293.
- Denenberg, V. H. (1983). Lateralization of function in rats. *Am. J. Physiol.* **245**, R505–R509.
- Denenberg, V. H. (1984). Some statistical and experimental considerations in the use of the analysis-of-variance procedure. *Am. J. Physiol.* **246**(4 Pt 2), R403–R408.
- Denenberg, V. H., Hofmann, M., Garbanati, J. A., Sherman, G. F., Rosen, G. D., and Yutzy, D. A. (1980). Handling in infancy, taste aversion, and brain laterality in rats. *Brain Res.* **200**, 123–133.
- Diaz, R., Sokoloff, P., and Fuxe, K. (1997). Codistribution of the dopamine D3 receptor and glucocorticoid receptor mRNAs during striatal prenatal development in the rat. *Neurosci. Lett.* **227**, 119–122.
- Diorio, D., Viau, V., and Meaney, M. J. (1993). The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *J. Neurosci.* **13**, 3839–3847.
- Drew, K. L., Lyon, R. A., Titeler, M., and Glick, S. D. (1986). Asymmetry in D-2 binding in female rat striata. *Brain Res.* **363**, 192–195.
- Fox, C., Merali, Z., and Harrison, C. (2006). Therapeutic and protective effect of environmental enrichment against psychogenic and neurogenic stress. *Behav. Brain Res.* **175**, 1–8.

- Froehlich, T. E., Lanphear, B. P., Dietrich, K. N., Cory-Slechta, D. A., Wang, N., and Kahn, R. S. (2007). Interactive effects of a DRD4 polymorphism, lead, and sex on executive functions in children. *Biol. Psychiatry* **62**, 243–249.
- Gasbarri, A., Campana, E., Pacitti, C., Hajdu, F., and Tömböl, T. (1991). Organization of the projections from the ventral tegmental area of Tsai to the hippocampal formation in the rat. *J. Hirnforsch.* **32**, 429–437.
- Geschwind, N., and Galaburda, A. M. (1985). Cerebral lateralization. Biological mechanisms, associations, and pathology: I. A hypothesis and a program for research. *Arch. Neurol.* **42**, 428–459.
- Grizenko, N., Shayan, Y. R., Polotskaia, A., Ter-Stepanian, M., and Joober, R. (2008). Relation of maternal stress during pregnancy to symptom severity and response to treatment in children with ADHD. *J. Psychiatry Neurosci.* **33**, 10–16.
- Guilarte, T. R., Toscano, C. D., McGlothlan, J. L., and Weaver, S. A. (2003). Environmental enrichment reverses cognitive and molecular deficits induced by developmental lead exposure. *Ann. Neurol.* **53**, 50–56.
- Hashimoto, K., Engberg, G., Shimizu, E., Nordin, C., Lindström, L. H., and Iyo, M. (2005). Elevated glutamine/glutamate ratio in cerebrospinal fluid of first episode and drug naive schizophrenic patients. *BMC Psychiatry* **5**, 6.
- Hausmann, M., and Güntürkün, O. (2000). Steroid fluctuations modify functional cerebral asymmetries: The hypothesis of progesterone-mediated inter-hemispheric decoupling. *Neuropsychologia* **38**, 1362–1374.
- Hirnstein, M., Leask, S., Rose, J., and Hausmann, M. (2010). Disentangling the relationship between hemispheric asymmetry and cognitive performance. *Brain Cogn.* **73**, 119–127.
- Jett, D. A., Kuhlmann, A. C., Farmer, S. J., and Guilarte, T. R. (1997). Age-dependent effects of developmental lead exposure on performance in the Morris water maze. *Pharmacol. Biochem. Behav.* **57**, 271–279.
- Keune, P. M., Schönenberg, M., Wyckoff, S., Mayer, K., Riemann, S., Hautzinger, M., and Strehl, U. (2011). Frontal alpha-asymmetry in adults with attention deficit hyperactivity disorder: Replication and specification. *Biol. Psychol.* **87**, 306–310.
- King, S., and Laplante, D. P. (2005). The effects of prenatal maternal stress on children's cognitive development: Project Ice Storm. *Stress* **8**, 35–45.
- Klur, S., Muller, C., Pereira de Vasconcelos, A., Ballard, T., Lopez, J., Galani, R., Certa, U., and Cassel, J. C. (2009). Hippocampal-dependent spatial memory functions might be lateralized in rats: An approach combining gene expression profiling and reversible inactivation. *Hippocampus* **19**, 800–816.
- Kuhlmann, A. C., McGlothlan, J. L., and Guilarte, T. R. (1997). Developmental lead exposure causes spatial learning deficits in adult rats. *Neurosci. Lett.* **233**, 101–104.
- Lange, N., Dubray, M. B., Lee, J. E., Froimowitz, M. P., Froehlich, A., Adluru, N., Wright, B., Ravichandran, C., Fletcher, P. T., Bigler, E. D., et al. (2010). Atypical diffusion tensor hemispheric asymmetry in autism. *Autism Res.* **3**, 350–358.
- Lanphear, B. P., Hornung, R., Khoury, J., Yolton, K., Baghurst, P., Bellinger, D. C., Canfield, R. L., Dietrich, K. N., Bornschein, R., Greene, T., et al. (2005). Low-level environmental lead exposure and children's intellectual function: An international pooled analysis. *Environ. Health Perspect.* **113**, 894–899.
- Laplante, D. P., Barr, R. G., Brunet, A., Galbaud du Fort, G., Meaney, M. L., Saucier, J. F., Zelazo, P. R., and King, S. (2004). Stress during pregnancy affects general intellectual and language functioning in human toddlers. *Pediatr. Res.* **56**, 400–410.
- Leonard, C. M., and Eckert, M. A. (2008). Asymmetry and dyslexia. *Dev. Neuropsychol.* **33**, 663–681.
- Lim, S. Y., Doherty, J. D., McBride, K., Miller-Ihli, N. J., Carmona, G. N., Stark, K. D., and Salem, N., Jr. (2005). Lead exposure and (n-3) fatty acid deficiency during rat neonatal development affect subsequent spatial task performance and olfactory discrimination. *J. Nutr.* **135**, 1019–1026.
- Lo, Y. C., Soong, W. T., Gau, S. S., Wu, Y. Y., Lai, M. C., Yeh, F. C., Chiang, W. Y., Kuo, L. W., Jaw, F. S., and Tseng, W. Y. (2011). The loss of asymmetry and reduced interhemispheric connectivity in adolescents with autism: A study using diffusion spectrum imaging tractography. *Psychiatry Res.* **192**, 60–66.
- Lordi, B., Patin, V., Protais, P., Mellier, D., and Caston, J. (2000). Chronic stress in pregnant rats: Effects on growth rate, anxiety and memory capabilities of the offspring. *Int. J. Psychophysiol.* **37**, 195–205.
- Lui, C. C., Wang, J. Y., Tain, Y. L., Chen, Y. C., Chang, K. A., Lai, M. C., and Huang, L. T. (2011). Prenatal stress in rat causes long-term spatial memory deficit and hippocampus MRI abnormality: Differential effects of postweaning enriched environment. *Neurochem. Int.* **58**, 434–441.
- Majkutewicz, I., Cecot, T., Jerzemska, G., Myślińska, D., Plucińska, K., Trojnar, W., and Wrona, D. (2010). Lesion of the ventral tegmental area amplifies stimulation-induced Fos expression in the rat brain. *Brain Res.* **1320**, 95–105.
- Maliszewska-Scisło, M., and Trojnar, W. (1999). Augmentation of ventral tegmental area stimulation-induced feeding by both stimulation and lesion of the contralateral ventral tegmental area in the rat. *Acta Neurobiol. Exp. (Wars)*. **59**, 287–293.
- Motlagh, M. G., Katsovich, L., Thompson, N., Lin, H., Kim, Y. S., Scahill, L., Lombroso, P. J., King, R. A., Peterson, B. S., and Leckman, J. F. (2010). Severe psychosocial stress and heavy cigarette smoking during pregnancy: An examination of the pre- and perinatal risk factors associated with ADHD and Tourette syndrome. *Eur. Child Adolesc. Psychiatry* **19**, 755–764.
- Nigg, J. T., Knottnerus, G. M., Martel, M. M., Nikolas, M., Cavanagh, K., Karmaus, W., and Rappley, M. D. (2008). Low blood lead levels associated with clinically diagnosed attention-deficit/hyperactivity disorder and mediated by weak cognitive control. *Biol. Psychiatry* **63**, 325–331.
- Nigg, J. T., Nikolas, M., Mark Knottnerus, G., Cavanagh, K., and Friderici, K. (2010). Confirmation and extension of association of blood lead with attention-deficit/hyperactivity disorder (ADHD) and ADHD symptom domains at population-typical exposure levels. *J. Child Psychol. Psychiatry.* **51**, 58–65.
- Nottebohm, F. (1970). Ontogeny of bird song. *Science* **167**, 950–956.
- Nottebohm, F. (1971). Neural lateralization of vocal control in a passerine bird. I. Song. *J. Exp. Zool.* **177**, 229–261.
- Oades, R. D., and Halliday, G. M. (1987). Ventral tegmental (A10) system: Neurobiology. 1. Anatomy and connectivity. *Brain Res.* **434**, 117–165.
- Oertel-Knochel, V., and Linden, D. E. (2011). Cerebral asymmetry in schizophrenia. *Neuroscientist* **17**, 456–467.
- Petroff, O. A., Errante, L. D., Rothman, D. L., Kim, J. H., and Spencer, D. D. (2002). Glutamate-glutamine cycling in the epileptic human hippocampus. *Epilepsia* **43**, 703–710.
- Piazza, P. V., Rouge-Pont, F., Deroche, V., Maccari, S., Simon, H., and Le Moal, M. (1996). Glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission. *Proc. Natl. Acad. Sci. U.S.A.* **93**, 8716–8720.
- Pokora, M. J., Richfield, E. K., and Cory-Slechta, D. A. (1996). Preferential vulnerability of nucleus accumbens dopamine binding sites to low-level lead exposure: Time course of effects and interactions with chronic dopamine agonist treatments. *J. Neurochem.* **67**, 1540–1550.
- Rodriguez, M., Castellano, M. A., and Palarea, M. D. (1990). Interhemispheric regulation of dopaminergic ascending systems. *Life Sci.* **47**, 377–384.
- Rogers, L. J., Zucca, P., and Vallortigara, G. (2004). Advantages of having a lateralized brain. *Proc. Biol. Sci.* **271**(Suppl. 6), S420–S422.
- Rosen, G. D., Finklestein, S., Stoll, A. L., Yutzey, D. A., and Denenberg, V. H. (1984). Neurochemical asymmetries in the albino rat's cortex, striatum, and nucleus accumbens. *Life Sci.* **34**, 1143–1148.
- Schneider, J. S., Lee, M. H., Anderson, D. W., Zuck, L., and Lidsky, T. I. (2001). Enriched environment during development is protective against lead-induced neurotoxicity. *Brain Res.* **896**, 48–55.
- Schuepbach, D., Skotchko, T., Duschek, S., Theodoridou, A., Grimm, S., Boeker, H., and Seifritz, E. (2012). Gender and rapid alterations of hemispheric dominance during planning. *Neuropsychobiology* **66**, 149–157.

- Scott, L. L., and Nguyen, L. M. (2011). Geographic region of residence and blood lead levels in US children: Results of the National Health and Nutrition Examination Survey. *Int. Arch. Occup. Environ. Health* **84**, 513–522.
- Sesack, S. R., and Grace, A. A. (2010). Cortico-Basal Ganglia reward network: Microcircuitry. *Neuropsychopharmacology* **35**, 27–47.
- Shaw, P., Lalonde, F., Lepage, C., Rabin, C., Eckstrand, K., Sharp, W., Greenstein, D., Evans, A., Giedd, J. N., and Rapoport, J. (2009). Development of cortical asymmetry in typically developing children and its disruption in attention-deficit/hyperactivity disorder. *Arch. Gen. Psychiatry* **66**, 888–896.
- Sherman, G. F., Garbanati, J. A., Rosen, G. D., Hofmann, M., Yutzey, D. A., and Denenberg, V. H. (1983). Lateralization of spatial preference in the female rat. *Life Sci.* **33**, 189–193.
- Sherman, G. F., Garbanati, J. A., Rosen, G. D., Yutzey, D. A., and Denenberg, V. H. (1980). Brain and behavioral asymmetries for spatial preference in rats. *Brain Res.* **192**, 61–67.
- Shinohara, Y., Hosoya, A., Yamasaki, N., Ahmed, H., Hattori, S., Eguchi, M., Yamaguchi, S., Miyakawa, T., Hirase, H., and Shigemoto, R. (2012). Right-hemispheric dominance of spatial memory in split-brain mice. *Hippocampus* **22**, 117–121.
- Simpson, J., and Kelly, J. P. (2011). The impact of environmental enrichment in laboratory rats—behavioural and neurochemical aspects. *Behav. Brain Res.* **222**, 246–264.
- Simpson, J., and Kelly, J. P. (2012). An investigation of whether there are sex differences in certain behavioural and neurochemical parameters in the rat. *Behav. Brain Res.* **229**, 289–300.
- Sullivan, R. M. (2004). Hemispheric asymmetry in stress processing in rat prefrontal cortex and the role of mesocortical dopamine. *Stress* **7**, 131–143.
- Sullivan, R. M., Chehab, S. L., Dufresne, M. M., and Laplante, F. (2012). Role of sex in the neurochemical and neuroendocrine correlates of paw preference in the rat. *Neuroscience* **202**, 192–201.
- Sullivan, R. M., and Dufresne, M. M. (2006). Mesocortical dopamine and HPA axis regulation: Role of laterality and early environment. *Brain Res.* **1076**, 49–59.
- Sullivan, R. M., Dufresne, M. M., and Waldron, J. (2009). Lateralized sex differences in stress-induced dopamine release in the rat. *Neuroreport* **20**, 229–232.
- Sun, L., Zhao, Z. Y., Hu, J., and Zhou, X. L. (2005). Potential association of lead exposure during early development of mice with alteration of hippocampus nitric oxide levels and learning memory. *Biomed. Environ. Sci.* **18**, 375–378.
- Tecchio, F., Zappasodi, F., Tombini, M., Caulo, M., Vernieri, F., and Rossini, P. M. (2007). Interhemispheric asymmetry of primary hand representation and recovery after stroke: A MEG study. *Neuroimage* **36**, 1057–1064.
- Tian, L., Wang, J., Yan, C., and He, Y. (2011). Hemisphere- and gender-related differences in small-world brain networks: A resting-state functional MRI study. *Neuroimage* **54**, 191–202.
- Toga, A. W., and Thompson, P. M. (2003). Mapping brain asymmetry. *Nat. Rev. Neurosci.* **4**, 37–48.
- Trojniar, W., and Klejbor, I. (1999). Facilitatory effect of unilateral lesion of the ventral tegmental area on locomotor response to stimulation of the contralateral ventral tegmental area: Involvement of GABAergic transmission. *Brain Res.* **842**, 419–430.
- Vallortigara, G. and Rogers, L. J. (2005). Survival with an asymmetrical brain: advantages and disadvantages. *Behav. Brain Sci.* **28**, 575–589.
- Virgolini, M. B., Rossi-George, A., Lisek, R., Weston, D. D., Thiruchelvam, M., and Cory-Slechta, D. A. (2008a). CNS effects of developmental Pb exposure are enhanced by combined maternal and offspring stress. *Neurotoxicology* **29**, 812–827.
- Virgolini, M. B., Rossi-George, A., Weston, D., and Cory-Slechta, D. A. (2008b). Influence of low level maternal Pb exposure and prenatal stress on offspring stress challenge responsivity. *Neurotoxicology* **29**, 928–939.
- Vogel, J. J., Bowers, C. A., and Vogel, D. S. (2003). Cerebral lateralization of spatial abilities: A meta-analysis. *Brain Cogn.* **52**, 197–204.
- Ward, I. L., and Weisz, J. (1984). Differential effects of maternal stress on circulating levels of corticosterone, progesterone, and testosterone in male and female rat fetuses and their mothers. *Endocrinology* **114**, 1635–1644.
- Wisniewski, A. B. (1998). Sexually-dimorphic patterns of cortical asymmetry, and the role for sex steroid hormones in determining cortical patterns of lateralization. *Psychoneuroendocrinology* **23**, 519–547.
- Yang, J., Hou, C., Ma, N., Liu, J., Zhang, Y., Zhou, J., Xu, L., and Li, L. (2007). Enriched environment treatment restores impaired hippocampal synaptic plasticity and cognitive deficits induced by prenatal chronic stress. *Neurobiol. Learn. Mem.* **87**, 257–263.
- Yi, S. J., Masters, J. N., and Baram, T. Z. (1994). Glucocorticoid receptor mRNA ontogeny in the fetal and postnatal rat forebrain. *Mol. Cell. Neurosci.* **5**, 385–393.
- Zimmerberg, B., and Reuter, J. M. (1989). Sexually dimorphic behavioral and brain asymmetries in neonatal rats: Effects of prenatal alcohol exposure. *Brain Res. Dev. Brain Res.* **46**, 281–290.