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MiniReview

Lifetime Consequences of Combined Maternal Lead and Stress

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Abstract: Elevated lead (Pb) exposure and high stress both target low socio-economic status populations. Both also act on the hypothalamic–pituitary–adrenal (HPA) axis. Pb disrupts cognition through effects on the mesocorticolimbic dopamine pathway. Stress hormones act on this same pathway via the HPA axis. The fact that Pb and stress are likely interactive risk factors served as the rationale for a series of studies in our laboratory. These demonstrate that stress can modify Pb effects, that Pb can modify stress responsivity, and, notably, that Pb + stress effects can occur in the absence of an effect of either alone in rats. Furthermore, maternal only Pb exposure can permanently alter basal corticosterone levels, stress responsivity (i.e. permanent modification of HPA axis function) and brain catecholamines in offspring of both genders. Interactive effects of Pb + stress are not limited to early development: even Pb exposures initiated post-weaning alter basal corticosterone and stress responsivity. Outcomes differ in relation to gender, brain region, stressor and time of measurement, making Pb + stress interactions complex. Altered HPA axis function may serve as a mechanism for the behavioural and catecholaminergic neurotoxicity associated with Pb, as well as for the increased incidence of disease and dysfunctions associated with low socio-economic status. The permanent consequences of maternal only Pb exposure suggest that Pb screening programmes should include pregnant women at risk for elevated Pb exposure, and that stress should be considered as an additional risk factor. Pb + stress effects observed in the absence of either risk factor alone raise questions about the capacity of current hazard identification approaches to adequately identify human health risks posed by neurotoxicants.

The adverse effects of elevated lead (Pb) body burden on cognitive functions of children have been extensively documented [1]. Indeed, it has now been demonstrated that population reductions in IQ scores can occur at blood Pb concentrations even below those currently deemed to be 'levels of concern' (10 μg/dl) by the Centers for Disease Control and Prevention [2]. The environmental reality, though, is that Pb exposures, like any other chemical exposure, occur in the context of numerous other risk modifiers, including genetic, host and other environmental factors. Most studies of Pb effects, both in human populations and in animal models, however, examine the effects of Pb exposure in isolation from co-occurring risk factors [3]. Thus, to date, with the exception of recent studies of vulnerability conferred by some genetic polymorphisms, almost nothing is known about how other potential risk modifiers influence the impact of Pb on central nervous system functions, despite the potential of such an understanding to allow refinement of animal models, more focused cohort studies, enhancement of the understanding of mechanisms and of human health risk and further development of behavioral therapeutic strategies.

Indeed, one risk factor highly likely to interact with Pb exposure is psychological stress (fig. 1). Currently, the highest levels of Pb in the USA are sustained by underprivileged, low socio-economic status children (i.e. inner-city minority children). Low socio-economic status populations experience higher levels of diseases and disorders than do their affluent counterparts [4–8], a phenomenon that has been hypothesized to arise from higher levels of stress and corresponding chronic elevations of glucocorticoids through the actions of stress on the hypothalamic–pituitary–adrenal (HPA) axis, the system that coordinates the body's physiological response to stress [9–11]. Studies also demonstrate that Pb exposure *per se* can produce permanent changes in HPA axis function [12–17]. In addition, both Pb and stress act on brain mesocorticolimbic dopamine and glutamate systems [18–26], which may explain their common consequences, including cognitive dysfunction and attention deficits [4,27–33], because the mesocorticolimbic dopamine system has extensive interactions with the HPA axis.

Stress by itself is not uniformly a negative event. Indeed, the stress response is the body's physiological mechanism to respond to threatening events. Moreover, under some conditions, stress can have positive effects, resulting in improved learning or memory [34]. It is widely held, however,

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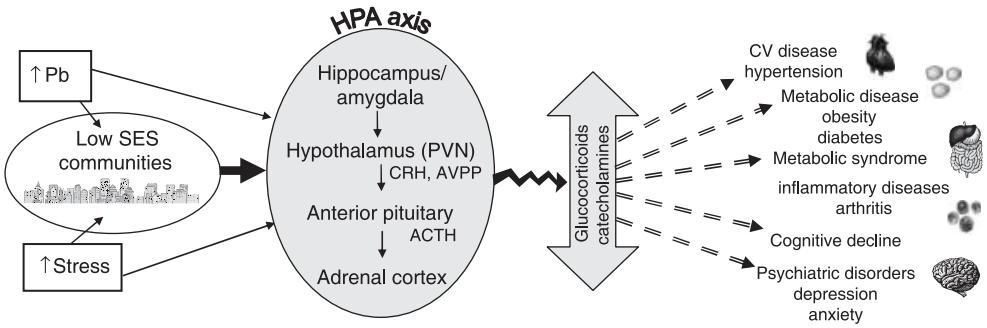

Fig. 1. Schematic depicting the rationale for the study of combined lead (Pb) and stress, as based on the fact that the highest levels of Pb exposure in the USA are sustained by low socio-economic status (SES) populations, and that the known higher levels of diseases and disorders experienced by those populations have been hypothesized to arise from higher levels of sustained stress associated with such environments. Stress is mediated via the HPA axis, and Pb exposure *per se* can influence HPA axis function. Such exposures, therefore, particularly when combined, can result in alterations in HPA axis function that will therefore potentially alter vulnerability for a host of diseases and disorders associated with HPA axis regulation.

that it is the balance of the stress load against the ability to respond and compensate (allostatic load) that is the ultimate arbiter of the consequences of the stressor [35].

Even while recognizing this potential positive component of stress, our studies as described here were premised on the fact that the 'normal' outcome of the stressors was that exhibited by control non-Pb treated, non-stressed rats, and, therefore, considered any deviation from that in Pb/stress-treated groups is considered as an aberrant response. Alterations in HPA axis function produced by Pb or combined Pb + stress, therefore, represent deviations from normal function. These can result in changes that include increases or decreases in functionality of the system through a wide variety of mechanisms. It is also important to note that HPA axis dysfunction through these means may have a wide range of physiological consequences in addition to effects on the central nervous system, given the broad scope of HPA hormonal activities (fig. 1). HPA axis dysfunction has been linked to a myriad of diseases and disorders in addition to cognitive impairment, including cardiovascular disease and hypertension, metabolic diseases such as obesity and diabetes, inflammatory-mediated diseases such as arthritis, and to psychiatric disorders that include depression and anxiety (fig. 1) [36]. Through its direct effects on the HPA axis, therefore, Pb could be directly contributing to the increased incidence of diseases and disorders in low socio-economic status communities.

Stress, of course, is also an inevitable component of human life, and one not experienced exclusively by low socio-economic status populations. Thus, the study of Pb + stress more accurately models the human condition in general, and corresponding results may have particular significance for understanding the true human health risks posed by Pb, especially because the cycles of poverty and elevated Pb are so congruous. Indeed, it can be asserted even more globally that a full understanding of the true risk posed by all environmental toxicants will ultimately require assessments of their interactions with other environmental and genetic risk factors.

The potential for Pb-stress interactions can be postulated in the context of a multihit model (i.e. a situation in which multiple risk factors target a common system of the brain), but act via different mechanisms. This is a scenario that may enhance vulnerability, particularly for the central nervous system [3], in that the brain may be readily able to compensate for the effects of an individual chemical or risk factor itself acting on a single specific target site of the brain, but when confronted by multiple insults at different functional sites (i.e. by different mechanisms), the system may no longer be able to evoke homeostatic mechanisms, thereby leading to sustained or cumulative damage.

Figure 2 depicts a hypothetical example of a multihit hypothesis of neurotoxicity featuring a mesolimbic dopamine terminal. Four concurrent insults all target the dopamine terminal, but do so by different mechanisms: insult A targets the vesicular monoamine transporter, insult B the enzyme monoamine oxidase, insult C attacks the enzyme converting tyrosine to 3,4-dihydroxyphenylalanine (DOPA), and insult D the dopamine transporter that takes dopamine back up from the synaptic cleft following its release. This multiplicity of insults occurring simultaneously at different sites within the system may constrict the range and flexibility of compensatory mechanisms, thereby compromising the integrity of the system. As a consequence, multiple risk factors acting at multiple target sites within a system could have effects that are more robust, more rapid in onset, or even differ in character from effects produced by a single risk factor or even by multiple risk factors that act at a single target site within a system.

This paper presents highlights from studies carried out by our laboratory examining Pb and stress interactions. Pb exposures in rats have repeatedly been shown to serve as a good model of Pb toxicokinetics and such exposures result in behavioural impairments that are highly comparable to those reported in children [29]. With respect to rodent models, a voluminous literature based on prenatal stress such as used in our studies demonstrates clears parallels to features of stress in human beings [35,37]. This paper includes

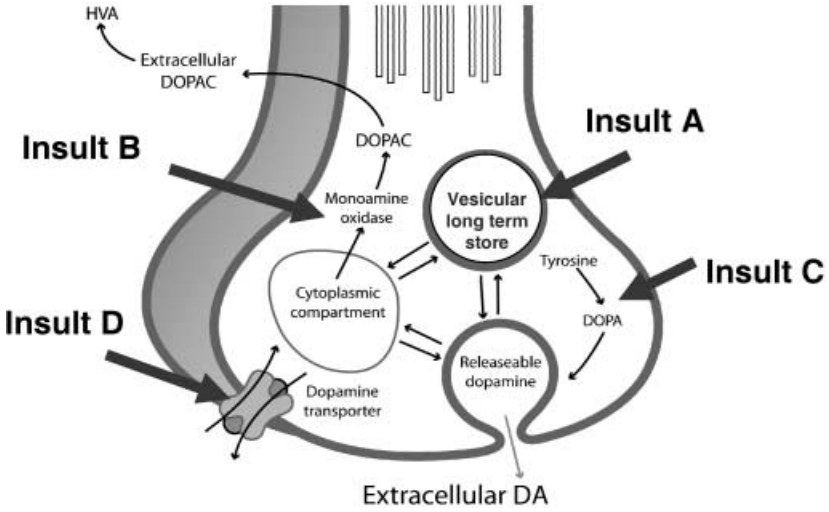

Fig. 2. Schematic depicting a multihit hypothesis as applied to a dopamine terminal within the central nervous system. Four concurrent insults are depicted that occur at different target sites of the dopamine terminal: insult A affecting the vesicular transporter; insult B affecting the metabolism of tyrosine to DOPA; insult C the enzyme monoamine oxidase; and insult D affecting the dopamine transporter. It is proposed that the brain may readily be able to compensate for the effects of an individual chemical itself acting on a particular system of the brain. However, when multiple target/mechanistic sites within that system are jointly affected, the system may no longer be able to adequately provoke homeostatic and repair mechanisms, thereby leading to sustained or cumulative damage [3].

a discussion of some generalities that have emerged from the findings to date, future research directions prompted by these findings, and their implications for understanding the human health hazards associated with Pb exposure.

In these studies, effects of Pb observed in Pb-treated, but not in Pb + stress-treated groups were defined an examples of modulation of Pb effects by stress. Conversely, effects observed in stress-treated, but not in Pb + stress-treated groups were defined as a modulation of stress effects by Pb. Interactive effects of Pb and stress could be additive, synergistic or potentiated. Additive effects were those consistent with combined effects of Pb alone and stress alone; synergistic effects exceeded additive effects.

Most importantly, potentiated effects were defined as effects of combined Pb + stress in the absence of effects of either variable alone. Potentiated effects are consistent with silent and cumulative toxicity of developmental Pb exposures, unmasked only by the addition of another insult, here, stress and can be consistent with the multihit hypothesis described above. Potentiated effects are particularly troubling when considered in the context of human health risk assessment strategies, because the paradigms typically employed for hazard identification generally study single risk factors in isolation, and, therefore, cannot detect such silent toxicities. Current hazard identification strategies for neurotoxicity are therefore likely to only be identifying the 'low-hanging fruit' [3].

Defining critical periods of Pb exposure and stress

A critical consideration related to studying potential interactions of Pb and stress is the role of developmental periods of Pb exposure and of stress treatment. Our initial studies [12,38] focused on the role of maternal Pb and maternal stress and the impact on offspring, including direct stress challenges to offspring. This was premised on the understanding that Pb exposure of the foetus derives from the mother, and that pregnant women, like all members of the population, can experience stress. Indeed, a substantial literature attests to the fact that prenatal stress exposure is known to have permanent and adverse consequences for heart, lung, kidney, gut and brain of offspring [39,40].

It is also important to understand whether Pb exposures and stress initiated only after the major periods of developmental vulnerability have ended might also impact the outcomes associated with Pb exposures. Indeed, Pb exposures later in development have been shown to adversely and selectively affect learning processes [41] indicating that the cognitive impairments associated with Pb have a long-term window of vulnerability. For this reason, a subsequent study examined the effects of Pb exposure initiated post-weaning in the rat (beginning at 21 days of age) and continuing for the duration of the study [42]. The early postnatal period in rats is considered to be at a stage of development comparable to third trimester in human beings, because much rodent brain development occurs postnatally. By 21 days of age (i.e. at weaning for the rat), this development is largely completed [43].

In human populations, of course, Pb exposure begins *in utero* and is sustained across the lifetime. Moreover, stress can occur via the mother or any time following birth. This suggests that the most relevant model for human translation ultimately is a continuous Pb exposure model with stress to the mother and consequent offspring stress. Studies employing this approach are now ongoing in our laboratory.

All studies included assessments in offspring of basal corticosterone and changes in corticosterone in response to

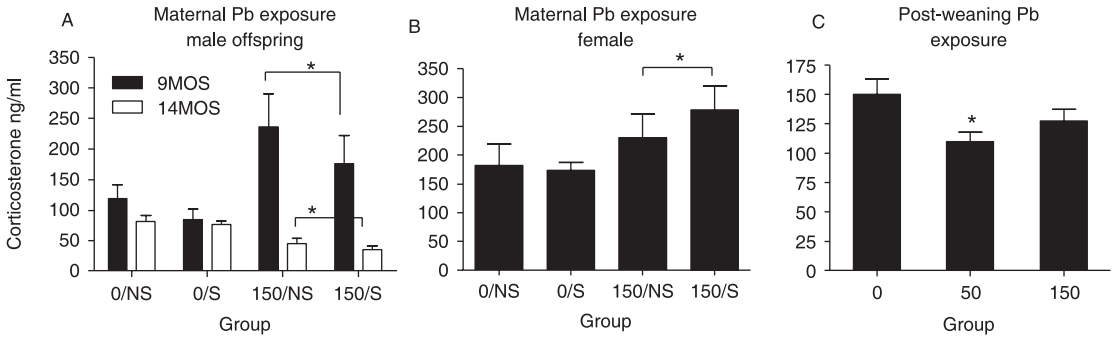

Fig. 3. Group mean ± S.E. basal corticosterone levels (ng/ml). Panel A: male offspring exposed via the dam to Pb, stress or the combination measured at either 9 or 14 months of age [47]. Panel B: female offspring exposed via the dam to Pb, stress or the combination at approximately 9 months of age [47]. Group designations for panels A and B are: 0/NS, no Pb, no stress; 0/S, maternal stress only; 150/NS, maternal Pb exposure only; 150/S, combined maternal Pb and stress. Panel C: male rats exposed to Pb at the designated concentrations (0, 50 or 150 p.p.m.) from 21 days of age, as measured after approximately 5 months of exposure [42]. * indicates significant difference between indicated groups (A and B) or from control (C).

various stress challenges. Changes in brain neurotransmitter systems, including dopamine and metabolites and serotonin and metabolites were measured in a variety of regions (frontal cortex, striatum, nucleus accumbens and hypothalamus) at various time-points. Behavioural changes were measured using a fixed-interval (FI) schedule of food reward. The FI schedule reinforces the first response occurring after a fixed interval of time has elapsed, with responses during the interval itself having no specified consequence. This schedule has a demonstrated history of sensitivity to Pb exposure, with increased rates of responding (which cannot accelerate the availability of reward) observed across a wide range of species and different exposure protocols at relevant blood Pb levels [44–46]. A series of various stress challenges were imposed in offspring at intermittent intervals, each prior to an FI behavioural test session (measured between 12 p.m. and 4 p.m. and during diestrous in females) to determine the impact of these challenges on FI performance. Three standard measures of FI performance were determined: (i) overall response rate (total number of responses divided by session time); (ii) post-reinforcement pause (time to the first response in the interval) and (iii) run rate (rate of responding after the post-reinforcement pause time has been subtracted out).

Highlights of outcomes from Pb and stress studies

Corticosterone changes.

Prior studies have reported effects of Pb on corticosterone levels (the rodent equivalent of cortisol), but at very high blood Pb levels [14–17]. Studies from our experiments completed to date reveal that: maternal only Pb exposure (which began 2 months prior to breeding and continued through lactation) resulted in permanent changes in corticosterone. As can be seen in fig. 3, maternal Pb exposure increased corticosterone levels of both male (panel A: 97–110%) and female (panel B: 27–58%) offspring, respectively, as measured at 9 months of age, well into the adult stage of the rat life cycle and long past the termination of Pb exposure (which ended at 21 days of age) [47]. No interactions of Pb by stress were detected, indicating that these effects were due to Pb and not modified by stress.

Importantly, the dynamic nature of maternal Pb exposure-induced changes in corticosterone function was revealed by additional determinations at 14 months of age in male offspring (unpublished data). At this time (fig. 3A, white bars), reductions in corticosterone were seen relative to levels at 9 months, but the decreases were significantly more pronounced in the Pb and Pb + stress groups (81–83% as compared to 8–33% in controls). Thus, Pb may also accelerate age-related decrements in basal corticosterone levels observed under these experimental conditions. These changes in corticosterone in response to maternal Pb are reminiscent of the permanent consequences of excess foetal glucocorticoids and prenatal stress on corticosterone and consistent with a foetal basis for numerous adult diseases associated with HPA axis dysfunction [39,40].

Another study in which chronic Pb exposure began only at 21 days of age indicated that Pb-associated changes in basal corticosterone levels are not limited to exposures occurring early in development (fig. 3C). Unlike the initial increases observed with maternal Pb treatment, exposures initiated post-weaning resulted in a U-shaped concentration–effect curve for corticosterone in male offspring (females were not used in this study) after 5 months of Pb exposure, with reductions of 27% at 50 p.p.m. and of 15% at 150 p.p.m. [42]. Corresponding blood Pb levels were 9–15 μg/dl and 23–27 μg/dl, respectively. The inverse U-shaped dose-effect curve is notable, given that many of the changes in neurotransmitter systems observed in non-stressed, Pb-treated animals likewise evidenced U-shaped or inverse U-shaped concentration effect functions, particularly for frontal cortex 5-hydroxyindoleacetic acid levels and nucleus accumbens 5-hydroxytryptamine (5-HT) levels and striatal DOPAC, homovanillic acid and 5-HT levels. Such curves are also potentially relevant to the recent findings that lower levels of Pb exposure (below 10 μg/dl) produce larger reductions in IQ than higher (>10 μg/dl) changes [2].

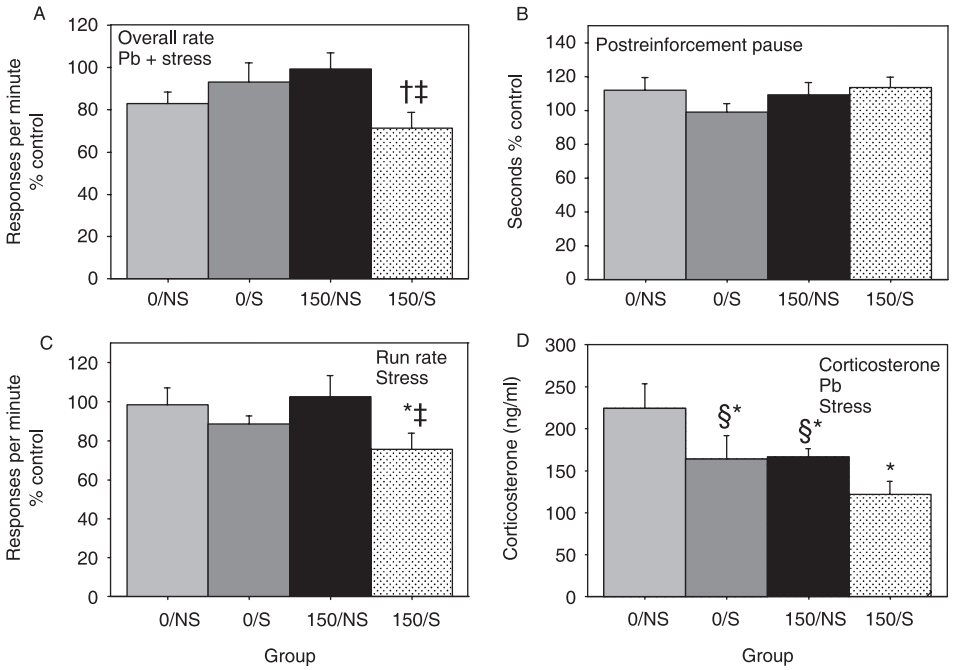

Fig. 4. Group mean ± S.E. fixed-interval (FI) performance measures and corticosterone levels following cold stress. Panel A shows overall response rate, panel B post-reinforcement pause time values, panel C run rate, and panel D corticosterone levels. Data for FI performance are plotted as percent of control, with the mean value of the two sessions preceding the restraint stress serving as control. Sample sizes for FI performance were n = 8 for 0/NS, n = 9 for 0/S, n = 7 for 150/NS and n = 10 for 150/S. Statistical analyses indicated: overall rate: Pb by stress interaction; run rate: main effect of stress; post-reinforcement pause time: no main effects or interactions. Corticosterone values are plotted as a percent of basal values. Absolute values for corticosterone following restraint stress were: 336.89 ± 33.3 for 0/NS, 271.12 ± 21.01 for 0/S; 320.87 ± 23.74 for 150/NS and 295.53 ± 26.3 for 150/S. Statistical analysis revealed a main effect of Pb and a main effect of stress for corticosterone values. * differs from 0/NS; † differs from 0/S; ‡ differs from 150/NS; § indicates marginal difference [38].

Numerous studies from our laboratory have shown the importance of mesocorticolimbic, dopaminergic and glutamatergic systems to the mediation of the changes in both learning [48] and FI performance [19] found in Pb-treated rats. These neurotransmitter systems have intricate and extensive interactions with the HPA axis [23,24,26]. Thus, alterations in the HPA axis and corticosterone induced by Pb could produce changes in mesocorticolimbic dopamine and glutamate function that contribute to these behavioural deficits. Alternatively, it may be that the changes in brain mesocorticolimbic systems are the primary site of damage by Pb that subsequently provokes HPA axis dysfunction. It is, of course, also possible that Pb exposures target both sites, and interactive effects occur.

Responsivity to stress challenges.

Alternations in response to stress challenges, measured as changes in FI performance from a previously stable baseline following the imposition of a stressor, were likewise seen in response to developmental Pb exposures, some of which were manifest as potentiated effects, that is, changes only observed under conditions of combined Pb + stress. For example, fig. 4 depicts changes in female offspring FI performance following cold stress (30 min. at 5°C) that reduced FI overall response rates (panel A) and run rates (panel C), without altering post-reinforcement pause times (panel B) with these effects observed only in Pb + stress offspring. Notably, these potentiated effects must reflect mechanisms in addition to alterations in the corticosterone response to cold stress, because corticosterone changes in the Pb + stress group appeared to be additive of those produced by Pb alone and stress alone (panel D). As measured well into adulthood, changes in both basal corticosterone (fig. 3) and stress responsivity (fig. 4) appear to be either extremely protracted or life-long consequences of combined maternal Pb and/or Pb + stress.

Post-weaning Pb exposure (i.e. exposure that began after major critical periods of development had ended), also altered stress responsivity (fig. 5). As previously observed in our studies, post-weaning Pb exposure itself increases FI response rates (bars indicated as 'baseline'), here in a concentration-related fashion [49,50]. In this case, novelty stress (30 min. measurement of activity in a locomotor activity device) imposed prior to the FI session blunted FI overall response rates of male offspring, but only in the Pb-exposed groups, an example of a type of silent Pb by stress interaction, because it was an effect of Pb only unmasked by a later stress challenge.

Changes in FI performance and brain neurochemistry.

To date, observed changes in basal FI performance and in brain neurochemistry in these Pb-stress interaction studies have been complex in nature, differing by developmental period of exposure, gender, time-point and brain region [12,42]. These

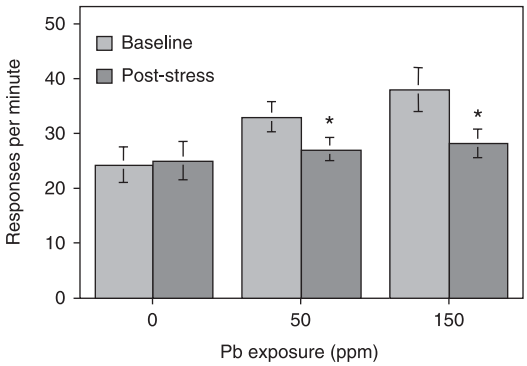

Fig. 5. Group mean ± S.E. fixed-interval (FI) overall response rates prior to (baseline) and after the imposition of novelty stress in male rats exposed to 0, 50 or 150 p.p.m. Pb acetate in drinking water from weaning. Novelty stress (measurement of locomotor activity) preceded an FI session during week 12 in all rats in each treatment group. Sample sizes were 12 per group. * indicates significant difference between indicated groups [42].

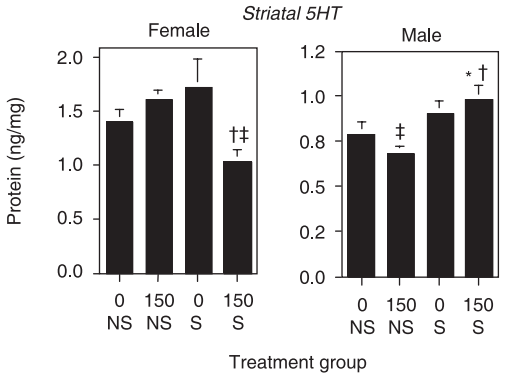

Fig. 7. Group mean ± S.E. concentrations of striatal 5-HT (ng/ml protein) in female (left) and male (right) offspring following maternal exposure to no lead or stress (0/NS), maternal stress (0/S), maternal Pb (150/NS) or to combined maternal Pb + stress (150/S) measured after the termination of behavioural testing. Sample sizes as defined in the legend for fig. 5. * differs from 0/NS; † differs from150/NS; ‡ differs from 0/S [12].

complexities significantly complicate the ability to relate behavioural and biochemical changes (i.e. to determine associated mechanisms of effect). At the same time, however, the multiplicity of neurochemical changes in response to Pb and stress permits a better understanding of the selectivity and specificity of any relationships that can be determined, and also allows evaluation of multiple mechanisms of action for behavioural and stress challenge responses.

Figures 6 and 7 show examples of potentiated effects of maternal Pb + stress in female and male offspring, respectively, brain neurotransmitter systems measured at approximately 9 months of age, following the termination of FI behavioural testing [12]. In frontal cortex (fig. 6), a region critical to learning and executive functions, an increase in dopamine of 30% was observed in female Pb + stress offspring (150/S), despite trends towards reductions in the Pb alone (150/NS) and stress alone (0/S) groups. Concurrently, a trend towards a reduction in levels of the dopamine metabolite DOPAC was found in the Pb + stress group relative to controls, collectively suggesting intracellular dopamine processing deficits. In contrast, no significant changes were found in males, where actually a trend towards a reduction rather than an increase was observed in the Pb + stress offspring.

Maternal Pb + stress also targets striatal 5-HT receptors/systems, an effect in this case occurring in both genders, although, again, resulting in changes in opposite directions in males and females (fig. 7). In females, despite trends towards increases in 5-HT levels in response to both Pb alone (150/NS) and to stress alone (0/S), a significant reduction of 23% occurred with Pb + stress (150/S), thus an effect of a direction opposite to that produced by either risk factor alone. In male offspring, reductions were seen in 5-HT levels in response to Pb alone, no significant changes were seen in

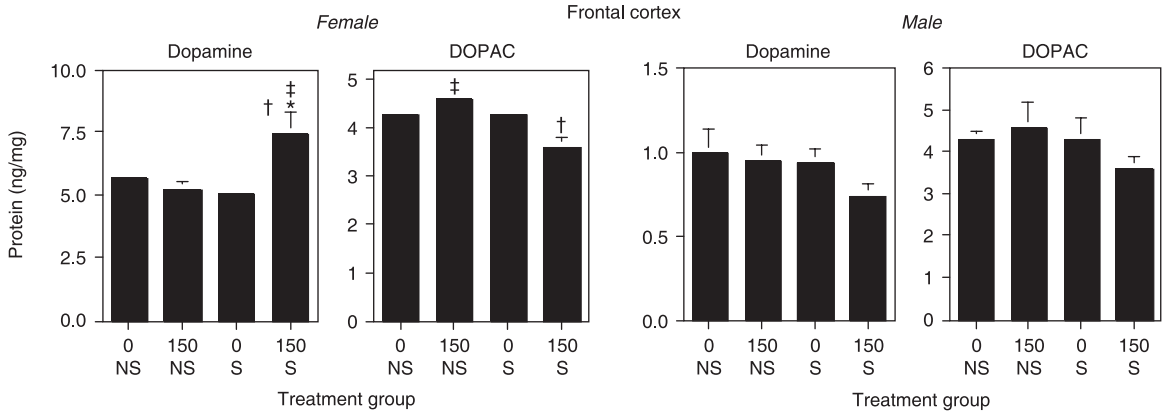

Fig. 6. Group mean ± S.E. concentrations of dopamine and DOPAC (ng/ml protein) in frontal cortex of female (left) and male (right) offspring following maternal exposure to no lead or stress (0/NS), maternal stress (0/S), maternal Pb (150/NS) or to combined maternal Pb + stress (150/S) measured after the termination of behavioural testing. Sample sizes for females: 0/NS = 8, 0/S = 11; 150/NS = 7, 150/S = 10. Sample sizes for males: 0/NS = 7, 0/S = 10; 150/NS = 8, 150/S = 8. * differs from 0/NS; † differs from150/NS; ‡ differs from 0/S [12].

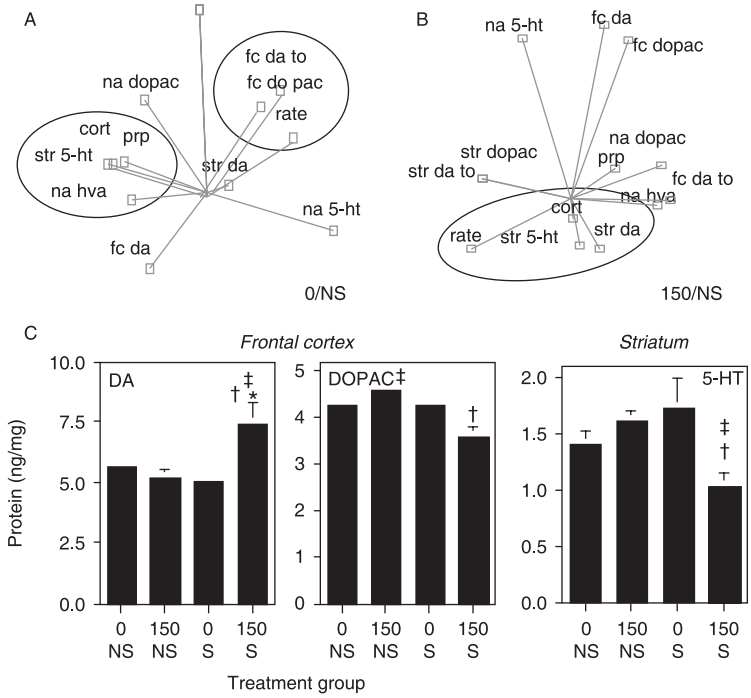

Fig. 8. Panels A and B: factor loading plots, respectively, for control (0/NS) and Pb + stress (150/S) female offspring showing first three components of principal component analysis, based on FI overall response rates and post-reinforcement pause times from week 8, basal corticosterone levels and adult levels of neurotransmitters that changed in response to Pb, stress or Pb + stress: frontal cortex DA, DOPAC and DA turnover, nucleus accumbens DA, HVA, and 5-HT and striatal DA, DOPAC, HVA, 5-HT and DA turnover. Abbreviations: cort = basal corticosterone; da to = dopamine turnover; nac = nucleus accumbens; prp = postreinforcement pause time; rate = mean overall response rate; str = striatal; hva = homovanillic acid. Sample sizes were 8 for the 0/NS group and 10 for the 150/S group. Panel C: as defined in legends for figs 6 and 7 for female offspring [12].

response to stress alone, but Pb + stress significantly increased 5-HT levels by 44%.

Findings with 5-HT systems are consistent with known interactions of serotonin and the HPA axis. Glucocorticoids can modify 5-HT receptor function and activity [51]. 5-HT neurons in brainstem express glucocorticoid receptors that are regulated by circulating corticosterone [52]. Moreover, administration of tricyclic antidepressants can suppress HPA activity [53].

Gender differences in outcomes related to prenatal stress and excess foetal glucocorticoids are well documented and suggest the role for oestrogen in modulation of stress effects [40]. It is important to note that current hazard identification strategies would have deemed Pb to be generally without effects on both dopamine and 5-HT systems, because no potential risk modifications are studied.

Relating behavioural effects to neurochemical and biochemical changes

As is clear from these examples, the consequences of Pb and stress vary markedly in response to developmental period of exposure, time of measurement, brain regions, behavioural baseline and gender, underscoring the difficulties in relating behavioural outcomes to biochemical and neurochemical alterations. Given the sizable number of outcome measures and multiple time-points in these experiments, we considered that principal component analysis might be used to initially demonstrate clustering among these outcome measures that could be indicative of mechanistic relationships.

An example of this approach is shown in fig. 8 comparing normal female offspring (0/NS) to those subjected to maternal Pb + stress (150/S) [12]. Panels A and B, respectively, show the outcomes of the corresponding principal component analysis for each group using FI response rates and post-reinforcement times (derived from week 8 of behavioural testing), basal corticosterone levels and neurotransmitter levels collected at the termination of behavioural testing [12]. In controls (panel A), FI response rates (rate) clustered with frontal cortex DOPAC (fc dopac) and dopamine turnover (fc da to), while post-reinforcement pause times (prp) clustered with nucleus accumbens HVA (na hva), and with striatal 5-HT (str 5-ht) and corticosterone (cort) levels. Moreover, the clustering of 5-HT and corticosterone is interesting in light of the well-known interactions of serotonergic systems with the HPA axis as described above. The clustering of FI response rate with frontal cortical dopamine function is consistent with studies from our laboratory showing the importance of mesocorticolimbic systems to this behaviour [20,54].

Following combined maternal Pb + stress (150/S, panel B), the clustering patterns change. FI response rates now cluster

with corticosterone, striatal 5-HT and striatal dopamine. Consistent with this outcome, corticosterone levels were elevated (fig. 3B), frontal cortical dopamine was altered, and striatal 5-HT levels were reduced (fig. 8, panel C) in Pb + stress females. These findings suggest frontal cortex dopamine and striatal 5-HT alterations as potential mechanisms in females of maternal Pb + stress mediation of FI performance, an assertion that can be further evaluated by manipulations of corresponding receptor systems alone or concurrently. It is notable in this regard that oral administration of a selective serotonin reuptake inhibitor during postnatal weeks 1–3 normalized the corticosterone response to stress, serotonin turnover in hippocampus, and the density of dendritic spines and synapses in the CA3 region of hippocampus in male offspring of mouse dams subjected to restraint stress three times daily from days 15 through 21 of gestation [55].

Future research needs and implications of Pb-stress interactions

Although it is already evident that studies of combined Pb and stress as co-occurring risk factors produce complex results that differ in relation to multiple experimental conditions, there are, nevertheless, some findings that have emerged that assist in focusing and guiding future research directions, understanding the true human health risks posed by Pb exposure, and modifying approaches now used to understand the risks posed by other toxicants.

General findings and future research directions

Pb exposure alone, both maternal and post-weaning, appears to permanently alter HPA axis function, effects that are observed in both genders and that are dynamic across time.

A future need is to determine the mechanism(s) by which Pb adversely targets HPA axis function. Determination of the specific window(s) during which maternal Pb + stress exposures result in HPA axis dysfunction may assist in refining the focus of potential mechanisms. Subsequent directions for both maternal and post-weaning exposures can include evaluation of the role of corticosterone changes through its normalization, and the use of genetically engineered mouse models to evaluate specific components of HPA axis function. In addition, it will be important in the case of maternal Pb exposures to determine whether this represents an epigenetic effect of Pb that is transgenerational.

Combined effects of Pb + stress occur in both genders, although the nature of the effects differs by gender.

The observed gender differences suggest a role for oestrogen in the mediation of both maternal and post-weaning effects of Pb + stress. Gender differences and female vulnerability to prenatal stress have been repeatedly described [40], but little is understood about gender differences and or mechanisms associated with gender differences in response to Pb [3]. Ovariectomy and oestrogen receptor antagonists will assist in addressing this issue.

Combined Pb + stress can produce potentiated effects.

Potentiated effects suggest support for the multihit hypothesis described above (fig. 2). If this is further confirmed, it would suggest the need to adopt this hypothesis more broadly in the evaluation of effects of neurotoxicants, using additional risk factors that target the same system(s) as Pb and stress to further determine whether the multihit scenario generally conveys greater vulnerability than 'additive effect' scenarios. Such an approach, moreover, should be more broadly adopted for human health risk assessment for neurotoxicology.

Vulnerability to combined Pb + stress in females may involve frontal cortical dopamine systems and striatal serotonin systems.

Changes in these neurotransmitter systems would be predicted to be important to the behavioural changes observed in our studies [12,38,42] and can be addressed using agonists/antagonists of various populations of receptors of these systems, particularly through microinjection approaches examining: (i) their ability to reverse effects of Pb and stress in treated animals, and (ii) their ability to provoke corresponding effects in normal animals.

Effects of combined Pb + stress occur at blood Pb levels as low as 10 μg/dl.

These results do not represent 'threshold' effects, but simply the lowest exposure levels yet studied in these models. However, they do suggest that uses of higher blood Pb levels are not required for future experiments, particularly when considered in the context of blood Pb levels relevant to adverse effects as currently understood. While continuous Pb exposure combined with both maternal and offspring stress represents the model most closely approximating human conditions, evaluation of different life stages may still be required to circumscribe periods of vulnerability and associated mechanisms.

Implications for risk assessment

Need to translate these findings to human populations.

If Pb exposure permanently alters HPA axis function in human beings, it would have tremendous implications for human health (fig. 1). First, it could suggest that Pb exposure actually contributes in an aetiological capacity to diseases and disorders associated with HPA axis dysfunction. It is already known that low socio-economic status populations (i.e. those that sustain the highest levels of Pb), also have higher levels of many diseases and dysfunctions [4–8]. Pb may thus contribute etiologically to these conditions. Support for such an assertion is provided by a recent study [56] showing the increase in relative hazard of stroke and myocardial infarction in individuals with blood Pb values as they increase between 1 and 10 μg/dl. Further support for the importance of Pb-stress interactions comes from a recent study reporting that the hypertensive effects of Pb exposure were further increased by high levels of stress

in male participants in the Normative Aging Study [57]. Prospective studies in children should also examine corticosterone and stress responsivity in relation to Pb exposures as a potential source of enhanced vulnerability.

Need to include pregnant women in screening for elevated blood Pb levels.

Our rodent studies show permanent effects of maternal only Pb and Pb + stress exposures on HPA axis function. Should these effects occur in human populations as well, it would suggest the need for blood Pb screening programmes to consider inclusion of pregnant women at higher risk of Pb exposure. Screening is now focused on young children, but permanent effects with broad physiological consequences may already have been passed along if the period of vulnerability is *in utero*.

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References

1 Lanphear BP, Hornung R, Khoury J et al. Low-level environmental lead exposure and children's intellectual function: an international pooled analysis. Environ Health Perspect 2005;**113**:894–9.
2 Canfield RL, Henderson CR, Cory-Slechta DA, Cox C, Jusko TA, Lanphear BP. Intellectual impairment in children with blood lead concentrations below 10 ug per deciliter. N Engl J Med 2003;**348**:1517–26.
3 Cory-Slechta DA. Studying toxicants as single chemicals: does this strategy adequately identify neurotoxic risk? Neurotoxicology 2005;**26**:491–510.
4 Anderson NB, Armstead CA. Toward understanding the association of socioeconomic status and health: a new challenge for the biopsychosocial approach. Psychosom Med 1995;**57**:213–25.
5 Dohrenwend BP. Socioeconomic status (SES) and psychiatry disorders: are the issues still compelling? Soc Psychiatry Psychiatr Epidemiol 1990;**25**:41–7.
6 Pappas G, Queen S, Hadden W, Fisher G. The increasing disparity in mortality between socioeconomic groups in the United States, 1960 and 1986. N Engl J Med 1993;**329**:103–9.
7 Starfield EL. Child health and social status. Pediatrics 1982;**69**:550–7.
8 Stipek JD, Ryan RH. Economically disadvantaged preschoolers: ready to learn but further to go. Dev Psychol 1997;**33**:711–23.
9 Vazquez DM. Stress and the developing limbic hypothalamic–pituitary–adrenal axis. Psychoneuroendocrinology 1998;**23**:663–700.
10 Lupien SJ, King S, Meaney MJ, McEwen BS. Can poverty get under your skin? Basal cortisol levels and cognitive function in children from low and high socioeconomic status. Dev Psychopathol 2001;**13**:653–76.
11 Munck A, Guyre PM, Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr Rev 1984;**5**:25–44.
12 Cory-Slechta DA, Virgolini MB, Thiruchelvam M, Weston DD, Bauter MR. Maternal stress modulates the effects of developmental lead exposure. Environ Health Perspect 2004;**112**:717–30.
13 Vyskocil A, Fiala Z, Ettlerova E, Tenjnorova I. Influence of chronic lead exposure on hormone levels in developing rats. J Appl Toxicol 1990;**10**:301–2.
14 Vyskocil A, Fiala Z, Lacinova V, Ettlerova E. A chronic study with lead acetate in female rats. J Appl Toxicol 1991;**11**:385–6.
15 Vyskocil A, Smejkalova J, Lacinova V. Dose-related stress reaction in male rats chronically exposed to lead acetate. Sb Ved Pr Lek Fak Karlovy Univerzity Hradci Kralove 1991;**34**:393–401.
16 Vyskocil A, Fiala Z, Tejnorova I, Tusl M. Stress reaction in developing rats exposed to 1% lead acetate. Sb Ved Pr Lek Fak Karlovy Univerzity Hradci Kralove 1991;**34**:287–95.
17 Vyskocil A, Fiala Z, Ettlerova E, Tejnorova I. Influence of chronic lead exposure on hormone levels and organ weights in developing rats. Sb Ved Pr Lek Fak Karlovy Univerzity Hradci Kralove 1991;**34**:275–85.
18 Barrot M, Marinelli M, Abrous DN, Rouge-Pont F, Le Moal M, Piazza PV. The dopaminergic hyper-responsiveness of the shell of the nucleus accumbens is hormone-dependent. Eur J Neurosci 2000;**12**:973–9.
19 Cory-Slechta DA, O'Mara DJ, Brockel BJ. Nucleus accumbens dopaminergic mediation of fixed interval schedule-controlled behavior and its modulation by low-level lead exposure. J Pharmacol Exp Ther 1998;**286**:794–805.
20 Cory-Slechta DA, Pazmino R, Bare C. The critical role of the nucleus accumbens dopamine systems in the mediation of fixed interval schedule-controlled operant behavior. Brain Res 1997;**764**:253–6.
21 Diorio D, Viau V, Meaney MJ. The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. J Neurosci 1993;**13**:3839–47.
22 Lowy M, Gault L, Yammamato B. Adrenalectomy attenuates stress induced elevation in extracellular glutamate concentration in hippocampus. J Neurosci 1993;**61**:1957–60.
23 Moghaddam B. Stress activation of glutamate neurotransmission in the prefrontal cortex: implications for dopamine-associated psychiatric disorders. Biol Psychiatry 2002;**51**:775–87.
24 Piazza PV, Rouge-Pont F, Deroche V, Maccari S, Simon H, Le Moal M. glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission. Proc Natl Acad Sci USA 1996;**93**:8716–20.
25 Pokora MJ, Richfield EK, Cory-Slechta DA. 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 1996;**67**:1540–50.
26 Rouge-Pont F, Deroche V, Le Moal M, Piazza PV. Individual differences in stress-induced dopamine release in the nucleus accumbens are influenced by corticosterone. Eur J Neurosci 1998;**10**:3903–7.
27 Bellinger DC, Hu H, Titlebaum L, Needleman HL. Attentional correlates of dentin and bone lead levels in adolescents. Arch Environ Health 1994;**49**:98–105.
28 Bradley RH, Corwyn RF. Socioeconomic status and child development. Annu Rev Psychol 2002;**53**:371–99.
29 Cory-Slechta DA. Relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic and glutamatergic neurotransmitter system functions. Annu Rev Pharmacol Toxicol 1995;**35**:391–415.
30 Dietrich KN, Ris MD, Succop PA, Berger OG, Bornschein R. Early exposure to lead and juvenile delinquency. Neurotoxicol Teratol 2001;**23**:511–8.
31 Needleman HL, Riess JA, Tobin MJ, Biesecker GE, Greenhouse JB. Bone lead levels and delinquent behavior. J Am Med Assoc 1996;**275**:363–9.
32 Schwartz J. Low-level lead exposure and children's IQ: a meta-analysis and search for a threshold. Environ Res 1994;**65**:42–55.
33 Dohrenwend BP. Social status and stressful life events. J Pers Soc Psychol 1973;**28**:225–35.

34 Zheng G, Zhang X, Chen Y, Zhang Y, Luo W, Chen J. Evidence for a role of GABAA receptor in the acute restraint stress-induced enhancement of spatial memory. Brain Res 2007;**1181**:61–73.
35 McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev 2007;**87**:873–904.
36 Seckl JR, Holmes MC. Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. Nat Clin Pract Endocrinol Metab 2007;**3**:479–88.
37 Lupien SJ, Fiocco A, Wan N et al. Stress hormones and human memory function across the lifespan. Psychoneuroendocrinology 2005;**30**:225–42.
38 Virgolini MB, Bauter MR, Weston DD, Cory-Slechta DA. Permanent alterations in stress responsivity in female offspring subjected to combined maternal lead exposure and/or stress. Neurotoxicology 2006;**27**:11–21.
39 Bowman RE, MacLusky NJ, Sarmiento Y, Frankfurt M, Gordon M, Luine VN. Sexually dimorphic effects of prenatal stress on cognition, hormonal responses, and central neurotransmitters. Endocrinology 2004;**145**:3778–87.
40 Weinstock M. Gender differences in the effects of prenatal stress on brain development and behaviour. Neurochem Res 2007;**32**,1730–40.
41 Cohn J, Cox C, Cory-Slechta DA. The effects of lead exposure on learning in a multiple repeated acquisition and performance schedule. Neurotoxicology 1993;**14**:329–46.
42 Virgolini MB, Chen K, Weston DD, Bauter MR, Cory-Slechta DA. Interactions of chronic lead exposure and intermittent stress: consequences for brain catecholamine systems and associated behaviors and HPA axis function. Toxicol Sci 2005;**87**:469–82.
43 Rice D, Barone S Jr. Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. Environ Health Perspect 2000;**108** (Suppl 3):511–33.
44 Cory-Slechta DA. The behavioral toxicity of lead: problems and perspectives. In: Barrett JE (ed.). Advances in Behavioral Pharmacology, vol. 4. Academic Press Inc., New York, 1984;211–55.
45 Cory-Slechta DA. Schedule-controlled behavior in neurotoxicology. In: Mitchell CL (ed.). Neurotoxicology, Target Organ Toxicology Series. Raven Press, New York, 1992;271–94.
46 Cory-Slechta DA, Weiss B. Alterations in schedule-controlled behavior of rodents correlated with prolonged lead exposure. In: Balster RL (ed.). Behavioral Pharmacology: The Current Status. Alan R. Liss, New York, 1985;487–501.
47 Cory-Slechta DA, Virgolini MB, Thiruchelvam M, Weston DD, Bauter MR. Maternal stress modulates effects of developmental lead exposure. Environ Health Perspect 2004;**112**:717–30.
48 Cory-Slechta DA, O'Mara DJ, Brockel BJ. Learning versus performance impairments following regional administration of MK-801 into nucleus accumbens and dorsomedial striatum. Behav Brain Res 1999;**102**:181–94.
49 Cory-Slechta DA, Thompson T. Behavioral toxicity of chronic postweaning lead exposure in the rat. Toxicol Appl Pharmacol 1979;**47**:151–9.
50 Cory-Slechta DA, Weiss B, Cox C. Performance and exposure indices of rats exposed to low concentrations of lead. Toxicol Appl Pharmacol 1985;**78**:291–9.
51 Azmitia EC Jr., McEwen BS. Adrenalcortical influence on rat brain tryptophan hydroxylase activity. Brain Res 1974;**78**:291–302.
52 Koenig JI, Gudelsky GA, Meltzer HY. Stimulation of corticosterone and beta-endorphin secretion in the rat by selective 5-HT receptor subtype activation. Eur J Pharmacol 1987;**137**:1–8.
53 Shimoda K, Yamada N, Ohi K, Tsujimoto T, Takahashi K, Takahashi S. Chronic administration of tricyclic antidepressants suppresses hypothalamo-pituitary-adrenocortical activity in male rats. Psychoneuroendocrinology 1988;**13**:431–40.
54 Evans SB, Cory-Slechta DA. Prefrontal cortical manipulations alter the effects of intra-ventral striatal dopamine antagonists on fixed-interval performance in the rat. Behav Brain Res 2000;**17**:45–58.
55 Ishiwata H, Shiga T, Okado N. Selective serotonin reuptake inhibitor treatment of early postnatal mice reverses their prenatal stress-induced brain dysfunction. Neuroscience 2005;**133**:893–901.
56 Menke A, Muntner P, Batuman V, Silbergeld EK, Guallar E. Blood lead below 0.48 micromol/l (10 microg/dl) and mortality among US adults. Circulation 2006;**114**:1388–94.
57 Peters JL, Kubzansky L, McNeely E et al. Stress as a potential modifier of the impact of lead levels on blood pressure: the normative aging study. Environ Health Perspect 2007;**115**:1154–9.

